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Research Article

Modulating Drug Release and Enhancing the Oral Bioavailability of Torcetrapib with Solid Lipid Dispersion Formulations

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Abstract. The development of drug dispersions using solid lipids is a novel formulation strategy that can help address the challenges of poor drug solubility and systemic exposure after oral administration. The highly lipophilic and poorly water-soluble drug torcetrapib could be effectively formulated into solid lipid microparticles (SLMs) using an anti-solvent precipitation strategy. Acoustic milling was subsequently used to obtain solid lipid nanoparticles (SLNs). Torcetrapib was successfully incorporated into the lipid matrix in an amorphous state. Spherical SLMs with mean particle size of approximately 15–18 μm were produced with high drug encapsulation efficiency (>96%) while SLNs were produced with a mean particle size of 155 nm and excellent colloidal stability. The *in vitro* drug release and the *in vivo* absorption of the solid lipid micro- and nanoparticles after oral dosing in rats were evaluated against conventional crystalline drug powders as well as a spray dried amorphous polymer dispersion formulation. Interestingly, the *in vitro* drug release rate from the lipid particles could be tuned for immediate or extended release by controlling either the particle size or the precipitation temperature used when forming the drug-lipid particles. This change in the rate of drug release was manifested *in vivo* with changes in Tmax as well. In addition, *in vivo* pharmacokinetic studies revealed a significant increase (~6 to 11-fold) in oral bioavailability in rats dosed with the SLMs and SLNs compared to conventional drug powders. Importantly, this formulation approach can be performed rapidly on a small scale, making it ideal as a formulation technology for use early in the drug discovery timeframe.

KEY WORDS: anti-solvent precipitation; controlled release; formulation; nanoparticles; solid lipid.

INTRODUCTION

Inadequate oral bioavailability and control over the rate of drug absorption has emerged as a significant challenge in the discovery and development of new drug candidates, particularly for poorly water-soluble drugs [1]. It is critically important to identify and address these challenges early in drug discovery to mitigate risks later in development. As a result, drug delivery approaches that can be applied to small scales are highly valuable. Among various formulation solubilization strategies (e.g., salt formation, complexation, amorphous dispersions, particle size reduction, etc.), lipid-based drug delivery systems are promising options, particularly for the increasingly large number of hydrophobic drug compounds. Lipids are known to enhance oral drug absorption [2–4] and can be prepared with low particle size [5], resulting in an increase of permeability and dissolution rate. In particular, solid lipid micro- and nanoparticles have attracted tremendous interest as alternative drug carriers to improve the bioavailability of poorly water-soluble molecules [6–9]. Such systems consist of a fat matrix based on naturally occurring lipids that are solid at both room and body temperature. Therefore, one advantage of solid lipid micro- and nanoparticles over polymer delivery systems is their generally good physiological tolerability. Compared to formulations made from liquid lipids (e.g., liposomes, emulsions), solid lipid formulations possess the additional advantages of better protecting incorporated drugs from degradation, improved physical stability, and the potential for modulating drug release from the solid matrix [10, 11].

A variety of methods have been reported to produce solid lipid micro- and nanoparticles, including high pressure homogenization [12, 13], ultrasonication [14, 15], microemulsification [16, 17], spray congealing [18], etc. Most of these techniques rely on dispersing the drug in molten lipid before processing into particles, a significant drawback when the solubility of the drug in the molten lipid phase is limited. Other alternatives to circumvent this limitation work by dispersing both drug and lipid in an organic solvent, which can then be mixed with an aqueous solution to create particles either through emulsification or precipitation. One promising example uses an anti-solvent precipitation method to generate drug solid

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dispersions. This approach is commonly employed to produce liposomes [19] and polymer nanoparticles [20, 21]. In addition to attaining high drug loading, this approach is also advantageous in that it does not involve the thermal and mechanical stresses associated with other methods, which can be detrimental to drug or product stability. The anti-solvent precipitation technique is based on precipitation of the drug and lipid dispersion from a water-miscible organic solution upon contact with the anti-solvent, typically an aqueous solution. Therefore, it is simple, fast, and does not require technically sophisticated equipment [22].

Torcetrapib was selected as a model compound in the present study for the development of solid lipid formulations. It is a highly lipophilic (ClogP=7.45) and very water insoluble molecule (solubility<40 ng/mL) developed for the treatment of atherosclerosis as a cholesteryl ester transfer protein (CETP) inhibitor [23]. In past clinical trials, torcetrapib was formulated with Miglyol 812 (a medium chain triglyceride excipient) in softgels. However, the dose was limited due to its poor solubility in Miglyol 812 and a pronounced positive food effect in humans [23]. As a result, torcetrapib is a well-suited model compound for the development of new formulation strategies to enhance oral exposure.

Herein, we report the development of novel formulations based on solid lipid dispersions for the purpose of improving the oral bioperformance of torcetrapib that could be performed on a small-scale suitable use during early drug discovery. Anti-solvent precipitation and acoustic milling were exploited to produce torcetrapib-loaded solid lipid micro- and nanoparticles, respectively. The lipid particles were comprehensively characterized for their physiochemical properties, including morphology, particles size, drug loading and encapsulation efficiency, and solid state of drug in the lipid matrix. The *in vitro* drug release behavior and *in vivo* drug oral absorption were investigated and evaluated against a spray dried amorphous polymer dispersion and crystalline drug powders as control formulations.

MATERIALS AND METHODS

Materials

Torcetrapib could be obtained from Sigma-Aldrich (St. Louis, MO, USA). Precirol ATO 5 (glyceryl palmitostearate) was received as a gift sample from Gattefossé (Paramus, NJ, USA). HPMCAS (hypromellose acetate succinate) LF grade was purchased from Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). Water used for formulations was filtered with a Millipore system. All other chemicals were of analytical grade and obtained commercially.

Production of Solid Lipid Microparticles Via Anti-Solvent Precipitation

Torcetrapib-loaded solid lipid microparticles (SLMs) were prepared by the anti-solvent precipitation method according to previous literature [22] with a slight modification. Twenty-five milligrams of drug and 100 mg of lipid were added to 1 mL isopropanol and heated to either 45 or 70°C until the solids were fully dissolved. Meanwhile, 10 mL of deionized water was heated to the same temperature. The organic solution was then injected into water under constant stirring at 500 rpm using a syringe equipped with 25 ½G gauge needle (Becton Dickinson, Franklin Lakes, NJ, USA). Stirring was continued at room temperature for 15 min. The pH of resulting slurry was adjusted to 1.5 by adding 0.1 M hydrochloric acid to induce flocculation of the precipitated particles. The aggregated particles were centrifuged at maximum speed on Centrific™ Centrifuge (Model 225, Fisher Scientific, Pittsburgh, PA, USA) for 20 min. The supernatant was then removed and the precipitate was washed two times with deionized water. The precipitated particles were isolated through lyophilization using a Labconco FreeZone Plus 2.5 L Benchtop freeze dryer (Labconco, Kansas city, MO, USA) overnight.

Production of Solid Lipid Nanoparticles Via Acoustic Milling

Torcetrapib-loaded solid lipid nanoparticles SLNs were prepared by further processing of the SLMs produced as described above via acoustic milling [24]. 260 mg of SLMs (2% particle loading) was weighed and added to a 30-mL glass jar together with 68.4 g of zirconia YTZ™ milling beads (500 μm, Tosoh Corp., Tokyo, Japan). Thirteen grams of a 2% Tween 80 solution was then added to the solids (particles and beads). The glass jar was then placed on a LabRAM mixer (Resodyn Acoustic Mixers, Inc., Butte, MT, USA) and mixed at 40% of intensity for 1 h.

Preparation of Torcetrapib-Polymer Dispersion Formulation by Spray Drying

Torcetrapib and HPMCAS-LF (drug/polymer ratio=1:4, 20% of drug loading) were weighed and dissolved in acetone. The solution was fed into a micro-spray dryer (ProCepT, Zelzate, Belgium) at a rate of 5 mL/min and sprayed into chamber from a nozzle with diameter of 0.8 mm. The atomization airflow rate was set at 4 L/min. The inlet temperature and outlet temperature of drying chamber was maintained at 76 and 50°C, respectively. The collected spray-dried particles were stored under vacuum overnight.

Scanning Electron Microscopy (SEM) Analysis

The morphology of the SLMs was studied using a Hitachi Scanning Electron Microscope S-3400 N (Hitachi Ltd., Tokyo, Japan). Approximately 1 mg of lyophilized particles was evenly sprinkled onto a stub with double-adhesive conductive tape. Samples were then sputter coated with gold under vacuum using a sputter coater. The images of particles were taken at an exciting voltage of 2.0 kV at a magnification of 1500×.

Particle Size, Distribution, and Zeta Potential

The particle size of the SLMs and drug-polymer dispersion solids were measured by laser diffraction (SYMPATEC, Sympatec Inc., Pennington, NJ, USA). The instrument is equipped with a dispensing unit RODOS/M, a vibratory feeder VIBRI, and a laser diffraction sensor HELOS. Dry powders were measured directly with a dispersing pressure of 1.0 bar and a laser measuring range of 0.5 to 350 μm.

The size and polydispersity of the SLNs were measured by dynamic light scattering (DynaPro Plate Reader, Wyatt Technology Corp., Santa Barbara, CA, USA), and the zeta potential of the SLNs was determined by phase analysis light scattering (Möbius, Wyatt Technology Corp., Santa Barbara, CA, USA) both at 25°C. Samples were diluted appropriately with deionized water before measurements.

HPLC Analysis of Torcetrapib

All analyses were performed on an Agilent 1100 system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a quaternary pump, a photodiode array UV-Vis detector, and an Ascentis Express C18 column (2.7 μm, 100×4.6 mm, Sigma-Aldrich, St. Louis, MO, USA). The mobile phase consisted of 0.1% phosphoric acid and acetonitrile. A gradient elution was utilized with acetonitrile increasing from 10 to 95% over 15 min at a flow rate of 1.0 mL/min. The column was heated at 40°C and the detection was carried out at 252 nm.

Drug Loading and Encapsulation Efficiency

SLMs

Approximately 2 mg of SLMs was weighed and dissolved in tetrahydrofuran (THF). The resultant solution was diluted appropriately and injected into HPLC for analysis of the drug concentration. Compared to standards of known concentrations, the drug loading was calculated as the amount of drug divided by the total mass of particles. The encapsulation efficiency was calculated as the actual drug loading divided by the theoretical drug loading (i.e., 20%).

SLNs

The encapsulation efficiency of torcetrapib in SLNs was determined by measuring the concentration of free drug in the dispersion medium (2% Tween 80). The encapsulation efficiency (EE) was calculated as:

$$EE\% = \left(1 - \frac{\text{concentration of free drug in supernatant}}{\text{concentration of total drug in dispersion}}\right) * 100\%$$

One milliliter of SLN dispersion was ultracentrifuged by Optima™ TLX Ultracentrifuge (Beckman Coulter, Inc., Brea, CA, USA) at 80,000 rpm for 20 min. The drug concentration in the supernatant was determined by HPLC and compared to standards of known concentrations. The concentration of total drug in dispersion was estimated by first diluting the dispersion with THF and then quantifying drug concentration by HPLC.

Modulated Differential Scanning Calorimetry Analysis

Modulated differential scanning calorimetry (mDSC) analyses of bulk torcetrapib, lipid, and the SLMs precipitated at 45 and 70°C were performed to characterize the solid state of torcetrapib in the lipid dispersions. A Q2000 DSC (TA instruments, New Castle, DE, USA) was used with a nitrogen purge at 50 mL/min. Samples were sealed in standard aluminum pans with lids and heated from 25 to 200°C. The modulation temperature amplitude was 1°C, the period was 1 min, and the underlying heating rate was 3°C/min.

X-ray Powder Diffraction Characterization

X-ray powder diffraction (XRPD) studies were performed using Philips X'pert X-ray diffractometer (PANalytical Inc., Westborough, MA, USA) on the same samples as above. The samples were exposed to CuK_α radiation as the X-ray source and scanned from 4 to 40° at a step scan mode of 0.06°/s. The operating voltage was 45 kV and current was 40 mA.

Colloidal Stability Evaluation of SLNs

The colloidal stability of SLNs was assessed by monitoring the particle size of SLNs stored at 4°C for 3 months. Additionally, the SLNs were diluted with water and simulated gastric fluid (SGF) (0.17% 10N HCl, 0.2% NaCl, pH=1.8) four times respectively and placed at 37°C chamber for 2 h, followed by examining the particle size by dynamic light scattering.

In Vitro Dissolution Studies

In vitro dissolution tests were conducted using dissolution testing apparatus (Distek Model 2100c, Distek, Inc., North Brunswick, NJ, USA) at 37°C. Approximately 3.2 mg of crystalline drug powders, spray-dried polymer dispersion, SLMs, and 300 μL of SLN dispersion were added to 50 mL of phosphate buffer (25 mM, pH 6.8) containing 0.17% of sodium dodecyl sulfate (SDS), respectively. The presence of SDS was sufficient to ensure that the solid samples were suitable wetted during the duration of the study. The dissolution medium was stirred with a paddle at 50 rpm. The experiments were performed under sink conditions. At predetermined time points, 400 μL of dissolution medium was withdrawn and ultracentrifuged at 80,000 rpm for 20 min. The supernatant was diluted appropriately and injected to HPLC for the analysis of drug concentration. At the end of study, 400 μL of the dissolution medium was obtained and diluted with THF to determine the total concentration of torcetrapib in the dissolution medium. The percentage of drug released was calculated as the concentration of torcetrapib in the supernatant divided by the total concentration of torcetrapib in the dissolution medium.

In Vitro Supersaturation Studies

In vitro supersaturation studies were performed using the same apparatus as in the dissolution studies at 37°C. An excess amount (approx. 12 mg) of crystalline drug powders, spray-dried polymer dispersion, and SLMs were added to 50 mL of phosphate buffer (25 mM, pH 6.8) containing 0.17% of SDS, respectively. The dissolution medium was stirred with a paddle at 50 rpm. After equilibrating for 24 h, 400 μL of dissolution medium was withdrawn and ultracentrifuged at 80,000 rpm for 20 min. The supernatant was diluted appropriately and injected into the HPLC for the analysis of torcetrapib concentration.

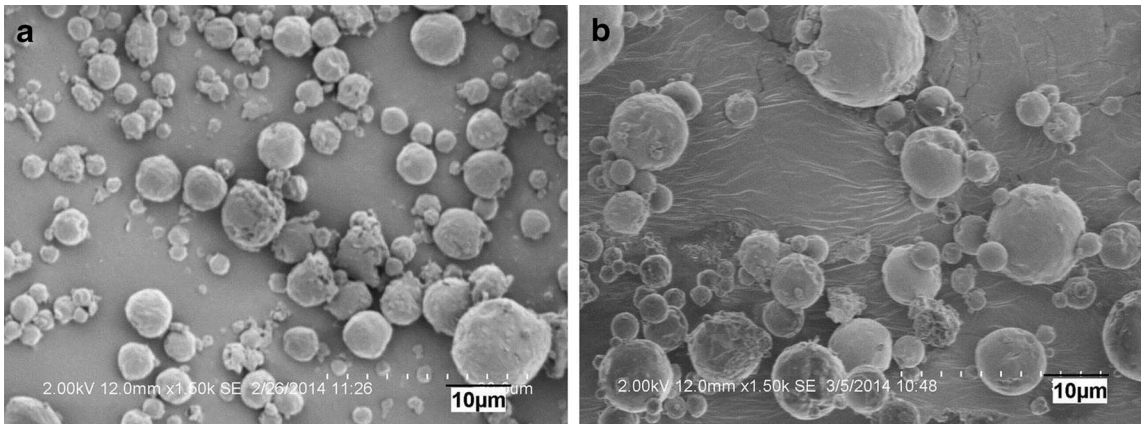

Fig. I. SEM images of SLMs precipitated at 45°C (**a**) and 70°C (**b**) after lyophilization

In Vivo Animal Studies

Oral Administration

Male Wistar-Han rats were used for oral administration studies. All animals were fasted overnight before dosing, provided water *ad libitum*, and fed 4 h following drug treatment. The fasted animals were orally given five torcetrapib formulations by gavage (n=3) at a torcetrapib dose of 10 mg/kg. The formulations include a suspension of crystalline torcetrapib powder in 0.5% of methyl cellulose (MC), a suspension of torcetrapib-polymer dispersion in 0.5% of acidified MC (5 mM HCl added to prevent dissolution of the polymer in the vehicle), suspensions of torcetrapib-loaded SLMs precipitated at 45 and 70°C, respectively, in 0.5% of MC, and an aqueous dispersion of torcetrapib-loaded SLNs in 2% Tween 80. Serial blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, 12, 18, and 24 h post-dose and placed into EDTA-containing tubes and centrifuged. Plasma was harvested and stored at -70°C until analysis. All animal procedures have been reviewed and approved by the Merck Research Laboratories (MRL)-Institutional Animal Care and Use Committee (IACUC).

Quantification of Plasma Concentration

Torcetrapib plasma concentrations were determined by LC-MS/MS following protein precipitation with acetonitrile. The LC-MS/MS system consisted of a Thermo Scientific LX2 autosampler equipped with two Transcend System pumps (Waltham, MA, USA) and an Applied Biosystems/MDS Sciex API 5000 mass spectrometer (Foster City, CA, USA). Chromatographic separation was achieved on an Waters Acquity HSS T3 column (1.8 µm, 2.1×50 mm, Milford, MA, USA) in conjunction with gradient conditions and mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). Mass spectrometric detection of the analytes was accomplished using the Turbo Spray interface operated in the positive ion mode. Analyte response was measured by multiple reaction monitoring (MRM) of transitions unique to each compound.

Calibration curves were generated and verified using standard and quality control samples prepared from an initial weighing of high purity compound. For the analysis of the plasma samples, standard samples were prepared by adding 10 µL of standard drug solutions in 1:1 acetonitrile:water (v:v) to 50 µL of control rat plasma with final concentrations ranging from 0.1 to 10,000 ng/mL. The limit of quantification was 1 ng/mL. The calibration curve was prepared by linear regression analysis of the plot of the peak area ratios of torcetrapib to internal standards against the nominal concentrations of torcetrapib. The equation of this curve was used to calculate the drug concentrations in all plasma samples.

Data Analysis

Pharmacokinetic parameters were calculated by established non-compartmental methods. The area under the plasma concentration *vs.* time curve from time zero to time t (AUC_{0-t}) was determined using the Watson software (version 7.3) with linear trapezoidal interpolation in the ascending slope and logarithmic trapezoidal interpolation in the descending slope.

Table I. Particle Size Distribution of SLMs

Formulation	Average (µm)	%10 (µm)	%50 (µm)	%90 (µm)
SLMs precipitated at 45°C	18.32	1.87	7.92	33.63
SLMs precipitated at 70°C	15.77	1.99	8.53	35.61

Table II. Drug Loading and Encapsulation Efficiency of SLMs

Formulation	Theoretical drug loading (%)	Actual drug loading (%)	Encapsulation efficiency (%)
SLMs precipitated at 45°C	20	19.2±0.8	96.4±5.4
SLMs precipitated at 70°C	20	19.3±1.1	95.8±4.1

Data are expressed as mean±S.D. (n=3)

Table III. Physicochemical Properties of Torcetrapib-Loaded SLNs

SLN formulations	Particle size (nm)	Polydispersity index (PI)	Zeta potential (-mV)	Encapsulation efficiency (%)
SLNs milled down from SLMs precipitated at 45°C	155.2±3.6	0.214±0.020	−28.6±1.2	73.8±0.4
SLNs milled down from SLMs precipitated at 70°C	154.8±0.2	0.238±0.001	−24.1±1.6	74.3±1.0

Data are expressed as mean±S.D. (n=3)

RESULTS AND DISCUSSION

Preparation of Torcetrapib-Loaded SLMs

The anti-solvent precipitation method was explored to produce the SLMs in the present study. This technique relies on the rapid diffusion of organic solvent into the aqueous phase upon injection, whereupon the high supersaturation of the lipid and drug initiates precipitation [22, 25]. This procedure could be performed on a small scale and does not require sophisticated equipment, making it ideal for use in the early drug discovery space where material may be limited. A prerequisite for solvent diffusion is miscibility between the solvent and aqueous phase at all ratios. Thus, the organic solvent was chosen to be isopropanol as suggested by previous literature [22]. However, heating to above 40°C was required in order to fully dissolve the lipid, Precirol ATO 5. In the present study, the solvent was controlled at two temperatures, 45 and 70°C, below and above the melting point of lipid (i.e., 55°C), respectively, to determine the effect on the final lipid dispersions. The isopropanol solution was then injected into aqueous media at a 10× dilution using a syringe which resulted in the rapid precipitation of the drug-lipid dispersion. Due to the small size as well as the low density of the precipitate, the produced particles were difficult to isolate using centrifugation. To overcome this limitation, the pH of the slurry was adjusted to 1.5 to promote flocculation and facilitate separation and isolation of particles by centrifugation [26].

After lyophilization to dry the particles, the precipitated SLMs were characterized using a scanning electron microscope for particle morphology. As seen in Fig. I, the particles were spherical in shape for both formulations produced at 45 and 70°C, respectively. The particle size and distribution were evaluated by laser diffraction. Similar particle size was achieved between the two SLMs (Table I). The drug loading and encapsulation efficiency of the lipid microparticles were also evaluated. The theoretical drug loading was 20% and the actual drug loading was determined to be 19.2±0.8 and 19.3±1.1 for the particles precipitated at 45 and 70°C respectively, giving a drug encapsulation efficiency around 96% for both formulations (Table II). This suggests that anti-solvent precipitation is a high-yielding procedure, since little drug was lost during the manufacturing process. Overall, there are no significant differences in product properties (e.g., morphology, size, and drug loading) between SLMs yielded from different precipitation temperatures.

Preparation of Torcetrapib-Loaded SLNs

Torcetrapib-loaded SLNs were produced from the torcetrapib-loaded SLMs using acoustic milling. Typically, low frequency acoustic energy was applied to the microparticles dispersed in a surfactant solution with milling media until the particles were milled to the desired size in the nanometer range [24]. This process, compared to traditional high shear milling methods, is mild, simple, efficient, and can be performed on small scale. In order to stabilize the particles against aggregation and thus efficiently stabilize the small particle size, an appropriate surfactant stabilizer at the appropriate concentration is required. After screening five non-ionic surfactants at four

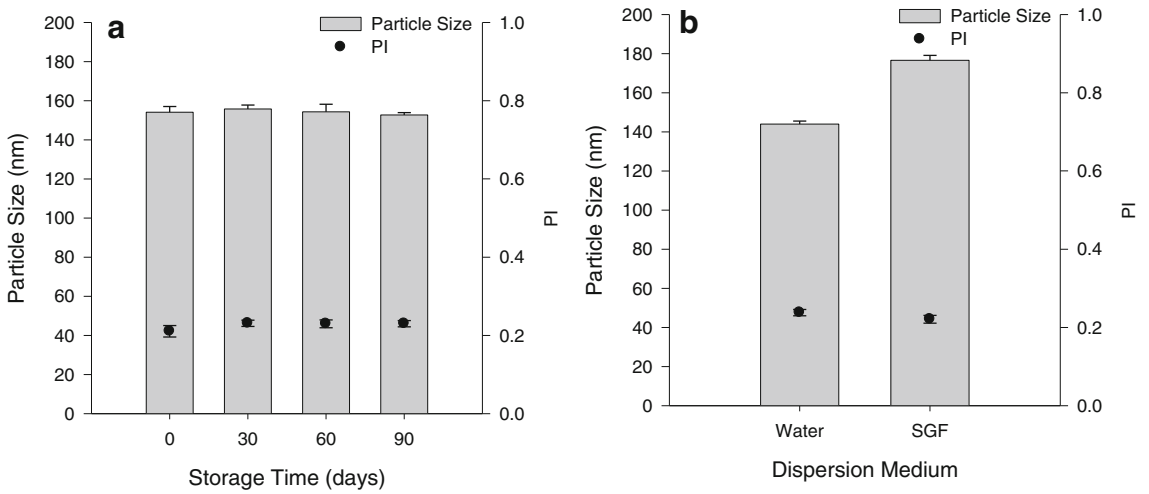

Fig. II. Average particle size and polydispersity index (PI) of torcetrapib-loaded SLNs under storage at 4°C for 3 months (**a**) and when dispersed in simulated gastric fluid (SGF) at 37°C for 2 h (**b**). Data are expressed as mean±S.D. (n=3)

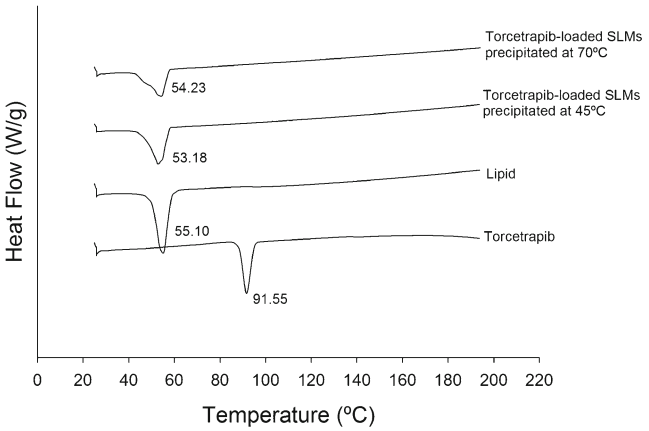

Fig. III. DSC thermograms of torcetrapib, lipid, and torcetrapib-loaded SLMs precipitated at 45 or 70°C

different concentration levels, Tween 80 at a 1:1 weight ratio to the microparticles was selected as the stabilizer giving the smallest nanoparticles (Supplemental Figure S1).

The physiochemical properties of the SLN formulations were comprehensively characterized in terms of particle size, polydispersity index (PI), zeta potential, and encapsulation efficiency (Table III). Drug-loaded SLNs milled from the SLMs precipitated from the two different temperatures were characterized by DLS. Both SLNs were around 155 nm in size, with a PI less than 0.3 indicating a narrowly distributed particle population [27]. Zeta potential, a key factor evaluating the physical stability of colloidal dispersions, measures the electrostatic value of the charged particle surface. The zeta potential values shown in Table III indicate that the electrostatic repulsion between particles will likely prevent particle aggregation and thus thermodynamically stabilize the nanoparticle dispersion. The encapsulation efficiency was around 74% for both SLN formulations. During the process of milling, particles reduced in size through attrition by acoustic energy, exposing a portion of the encapsulated drug to the Tween 80 dispersion medium, leading to some drug loss through solubilization. Considering that nanoparticles are dosed as an oral dispersion, the small loss of drug from particles into the medium was considered acceptable.

The colloidal stability of the torcetrapib-loaded SLNs was also investigated by evaluating the particle size and PI change during storage at 4°C for 3 months. Figure IIa suggests that no significant size or size distribution changes occur for the torcetrapib-loaded SLNs during this time. Another consideration when dosing SLNs orally is whether such system would be stabilized against the harsh *in vivo* gastric environment, as aggregation of particles *in vivo* prior to absorption would impact their bioperformance. Therefore, a study was carried out to assess the nanoparticle dispersion stability when dispersed into SGF at 37°C. As shown in Fig. IIb, only a slight yet acceptable particle size increase was observed, suggesting excellent colloidal stability of this delivery system after oral administration.

Solid State Characterization

Modulated DSC was performed to characterize the solid state of torcetrapib in the lipid dispersions. As shown in Fig. III, the melting peak of torcetrapib alone was observed at 91.55°C, matching that reported for the crystalline form [28]. The bulk lipid showed a melting endothermic peak at

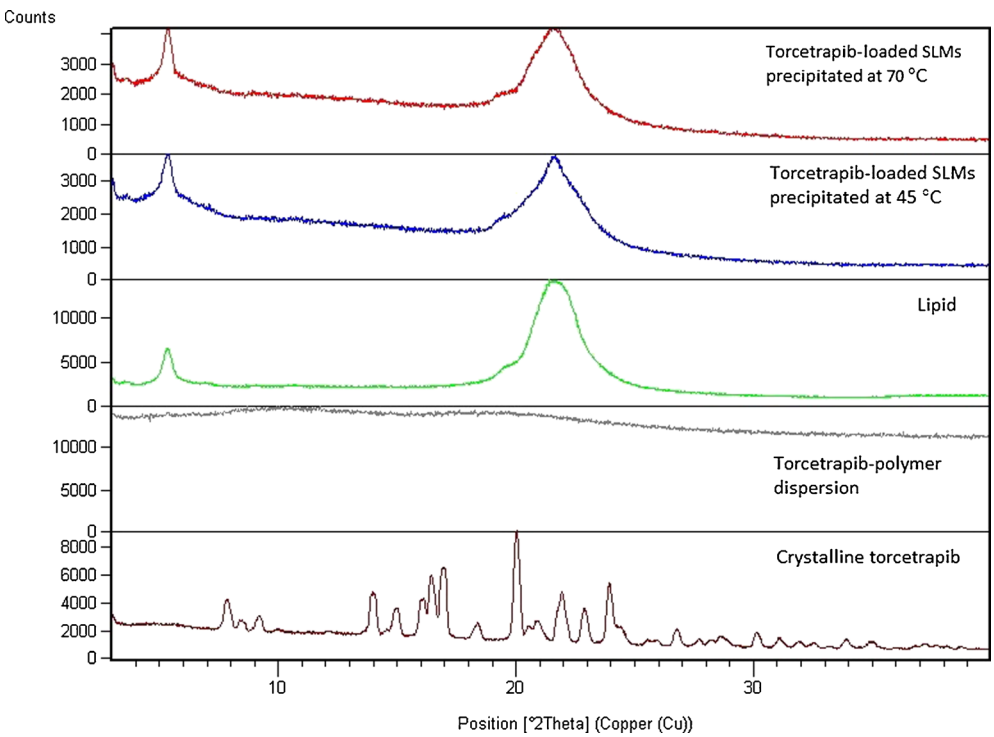

Fig. IV. Overlaid XRPD patterns of torcetrapib, lipid, torcetrapib-polymer dispersions, and torcetrapib-loaded SLMs precipitated at 45 or 70°C

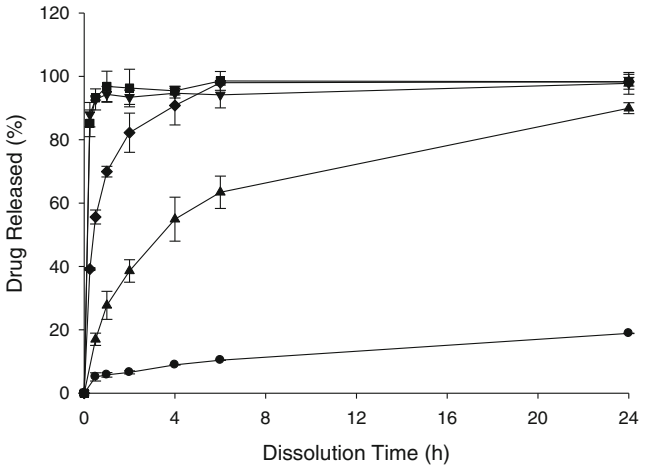

Fig. V. *In vitro* release of torcetrapib from crystalline torcetrapib powders (*black circle*), torcetrapib-polymer dispersion (*black square*), torcetrapib-loaded SLMs precipitated at 45°C (*black diamond*), torcetrapib-loaded SLMs precipitated at 70°C (*black up-pointing triangle*), and torcetrapib-loaded SLNs (*black down-pointing triangle*) in phosphate buffer (25 mM, pH 6.8) containing 0.17% SDS at 37°C. Symbols represent mean±S.D. (n=3). Torcetrapib-loaded SLNs were obtained from acoustic milling of torcetrapib-loaded SLMs precipitated at 70°C. No apparent difference in the drug release profiles was observed from SLNs that were milled down from SLMs precipitated either at 45 or 70°C (data not shown)

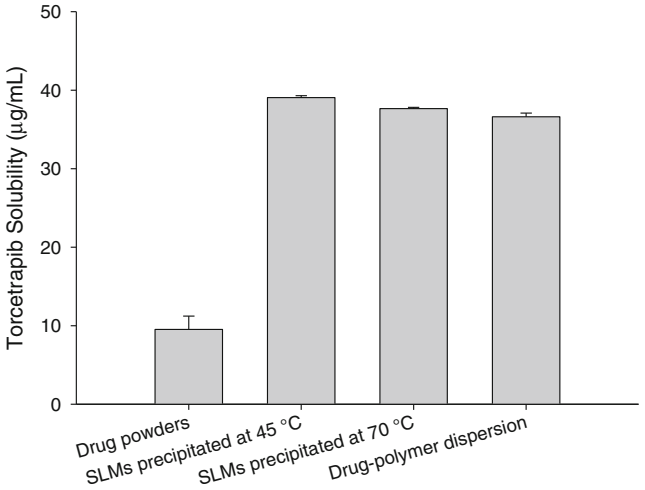

Fig. VI. *In vitro* apparent solubility of torcetrapib after 24 h dissolution at 37°C under supersaturated conditions. Data are expressed as mean±S.D. (n=3)

55.10°C. DSC thermograms of torcetrapib-loaded SLMs precipitated at either 45 or 70°C both displayed melting peaks very close to that of bulk lipid. However, the slight shift of peaks to 53.18 and 54.23°C and reduced enthalpy suggested the reduced crystallinity of lipid in the dispersions compared to the bulk lipid. The shoulder peak observed in SLMs precipitated at 70°C indicated the existence of low melting modifications of lipid polymorphs, resulting from the cooling of lipid during formulation [29]. The absence of the melting peak of torcetrapib indicated that torcetrapib may be dispersed in the lipid matrix in an amorphous form. One caveat, however, is the possibility of crystalline torcetrapib present dissolving in the molten lipid during the heating process of DSC measurement.

The XRPD data shown in Fig. IV confirmed the amorphous drug dispersion conclusions drawn from the mDSC measurements. Torcetrapib powder exhibited sharp peaks, indicating its crystalline nature. The identical positions of peaks with the bulk lipid and the disappearance of the characteristic peaks of torcetrapib in the drug-lipid particles at room temperature suggested that torcetrapib was incorporated into lipid matrix in a completely amorphous state. Since amorphous drugs usually are markedly more soluble than their crystalline counterparts, this behavior was expected to improve the apparent solubility of the drug in water and consequently result in increased oral bioavailability.

In Vitro Drug Release

The *in vitro* drug release behavior of the torcetrapib-loaded SLMs precipitated at two different temperatures, and the torcetrapib-loaded SLNs was investigated. Crystalline torcetrapib powders were also evaluated for drug dissolution as a control formulation. A spray-dried torcetrapib-polymer dispersion, an established technology for producing amorphous drug dispersions, was also studied as a control formulation [28].

As shown in Fig. V, torcetrapib powders exhibited a very slow release pattern (less than 20% drug released) during the 24 h study interval due to the limited crystalline solubility in the dissolution medium. The torcetrapib-polymer dispersion showed an immediate release with more than 90% of drug released during the first 30 min. This was expected since the drug was present in a more soluble amorphous state (confirmed by DSC and XRPD studies, data not shown) and the polymer is soluble in the aqueous medium, leading to immediate drug release and dissolution.

In contrast, torcetrapib-loaded SLMs precipitated at 45°C displayed a sustained drug release with an almost complete drug release within the first 6 h. By comparison, a much more extended drug release was observed in torcetrapib-loaded

Table IV. Results of Kinetic Model Fitting of Torcetrapib Release from Torcetrapib-Loaded SLMs Precipitated at 70°C

Kinetic model	Equations	Correlation coefficient (R^2)	Release rate constant (k)	Release exponent (n)
Zero order	*f vs. t*	0.7184	0.0303	–
First order	*lnf vs. t*	0.8321	0.2374	–
Higuchi	*f vs.* $t^{1/2}$	0.9325	0.1837	–
Korsmeyer-Peppas	*lnf vs. lnt*	0.9961	0.5251	0.49

f fractional drug released at time *t*, *t* dissolution time in hours

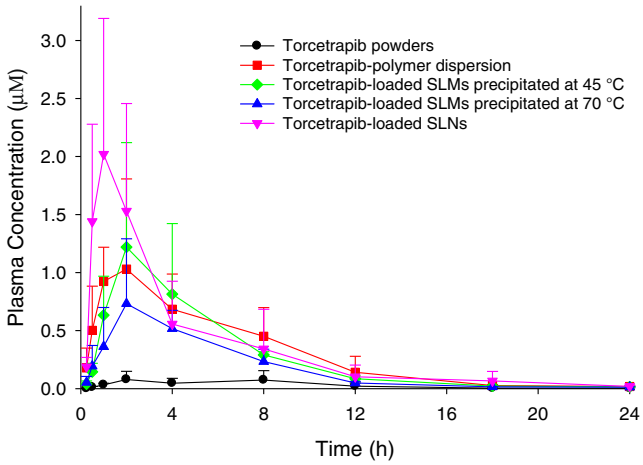

Fig. VII. Plasma concentration *vs.* time curves of torcetrapib in rats after oral administration of 10 mg/kg dose of torcetrapib formulations. Symbols represent mean±S.D. (n=3)

SLMs precipitated at 70°C, with 90% of drug being released only after 24 h. While quantitative drug release was observed with the two SLMs, different rates of sustained release of the drug could be realized in comparison to amorphous formulations made from hydrophilic polymers (e.g., spray-dried polymer dispersion). This difference in the rate of drug release was not due to differences in particle size, as the spray-dried drug-polymer dispersion had a very similar average particle size to the SLMs (see Supporting Information), but due to the nature of the polymer and lipid matrix.

Interestingly, this suggested that the rate of drug release could be easily tuned to obtain an extended release profile by simply controlling the processing temperature during the formation of the drug-lipid dispersions. The difference in the release rate between SLMs precipitated at two distinct temperatures is not likely due to surface area, as both SLMs revealed the same particle size distribution by laser diffraction (Table I). While the particle size was similar, differences in surface area or porosity of the particles could be potentially responsible for the observed differences in drug release rate. Alternatively, differences in lipid polymorphs or the uniformity of the distribution of drug within the particles could also be responsible. For example, at 70°C, the lipid exists in a liquid state and the drug may be preferentially dissolved in the molten lipid, resulting in drug encapsulated within the lipid matrix upon precipitation and solidification of the lipid. Differences in the cooling rate of the particles from 45 or 70°C may also affect the polymorphic state of the final particles recovered at RT. Further characterization is underway to determine the source of the differences in the rate of drug release of the SLMs.

Four kinetic models were applied to analyze the drug release kinetics of torcetrapib from the sustained release formulation, torcetrapib-loaded SLMs precipitated at 70°C (Table IV). The Korsmeyer-Peppas model with highest R^2 best described the mechanism of torcetrapib release from the SLMs. Since the release exponent n=0.49>0.43, the drug release from the spherical lipid matrix was governed by a non-Fickian transport mechanism [30].

Torcetrapib-loaded SLNs, on the other hand, displayed immediate drug release similar to that of the torcetrapib-polymer dispersion. This was expected due to the significantly higher surface area of the lipid nanoparticles and shorter drug diffusion length, resulting in an increase in dissolution rate. Additionally, drug exposed to the surface of the SLNs when the particles were milled down may also contribute to the immediate drug release. Under these conditions, the presence of Tween 80 in the SLN formulation was included only to stabilize the nanoparticles and did not change the solubility of the crystalline torcetrapib particles.

As a result, both immediate and controlled release profiles of torcetrapib could be achieved using solid lipid particles as a delivery vehicle by manipulating the particle size (nano- *vs.* microsize) or precipitation temperatures, enabling the preparation of oral formulations with well-tuned drug release profiles according to the compound's properties and therapeutic needs.

In Vitro Supersaturation Study

Creating stable amorphous dispersions is a well-recognized strategy for enhancing the oral absorption of poorly water-soluble drugs, as the amorphous form of a drug can allow for higher supersaturation of the metastable state relative to the crystalline equilibrium solubility [31]. However, excipients are often needed to inhibit the re-crystallization of the drug during the production and release process. Amorphous polymer dispersions have been extensively studied as a formulation approach for generating and stabilizing drug in amorphous state thus improving drug solubility for oral delivery [32–34].

In the current work, we evaluated the *in vitro* supersaturation of torcetrapib released from SLMs against a typical polymer dispersion after 24 h of equilibrium at 37°C. As shown in Fig. VI, comparable supersaturation level was achieved in both torcetrapib-loaded SLMs to the polymer dispersion formulation, which was ~4-fold greater relative to the intrinsic crystalline solubility of torcetrapib powders. Analysis of the particle size of the

Table V. Non-Compartmental Pharmacokinetic Parameters After Oral Administration of 10 mg/kg Dose of Torcetrapib Formulations in Rats

Formulation	C_{max} (μm)	t_{max} (h)	AUC $_{(0\text{-}24)}$ (μm*h)	AUC boost*
Crystalline torcetrapib powders	0.08±0.07	6.00±3.50	0.68±0.69	1.00
Torcetrapib-polymer dispersion	1.16±0.65	1.50±0.71	6.86±1.47	10.09
Torcetrapib-loaded SLMs precipitated at 45°C	1.22±0.90	2.00±0.00	6.19±3.40	9.10
Torcetrapib-loaded SLMs precipitated at 70°C	0.79±0.49	3.30±1.20	4.03±1.15	5.93
Torcetrapib-loaded SLNs	2.02±1.17	1.00±0.00	7.90±2.16	11.62

*Compared to mean AUC $_{(0\text{-}24)}$ of crystalline torcetrapib powders
Data are expressed as mean±S.D. (n=3)

SLMs dissolution medium suggested that submicron solubilized nanoparticles ~400 nm in diameter were formed during the dissolution process (Supplemental Figure S2). Such a species may be associated with a dissolved drug-lipid complex after release from the SLMs and is likely responsible for preventing the supersaturated drug solution from nucleating and precipitating. This result suggested that the solid lipid could stabilize the drug in a supersaturated state in solution, indicating its potential for increasing the *in vivo* bioavailability after oral administration.

In Vivo Pharmacokinetic Analysis

The *in vivo* absorption of torcetrapib after oral administration of torcetrapib-loaded solid lipid micro- and nanoparticles was investigated in rats at a dose level of 10 mg/kg. Crystalline drug powders and drug-polymer dispersion formulation were also evaluated as control formulations. The drug powders, drug-polymer dispersion, and drug-loaded SLMs were dosed as suspension in 0.5% of methylene cellulose. Torcetrapib-loaded SLNs were dosed as prepared as suspension in 2% of Tween 80 aqueous medium. The presence of 2% Tween 80 was required to stabilize the SLNs and was not expected to impact the solubility or absorption of torcetrapib based on our *in vitro* dissolution studies shown above.

The plasma concentration *vs.* time profiles following the oral administration of the different torcetrapib formulations in rats are shown in Fig. VII. The oral pharmacokinetic parameters are listed in Table V. The torcetrapib-polymer dispersion was used as a benchmark formulation for enhancing the drug's solubility and oral absorption, and the results showed an around 10-fold increases in $AUC_{0\text{-}24}$ compared to the crystalline drug powders. Torcetrapib-loaded SLMs precipitated at 45°C showed similar exposure to the polymer dispersion (6.19±3.40 *vs.* 6.86 ±1.47 in $AUC_{0\text{-}24}$). In comparison, a slight lower $AUC_{0\text{-}24}$ (4.03 ±1.15 *vs.* 6.19±3.40) was observed in torcetrapib-loaded SLMs precipitated at 70°C. This corresponds well with its 24-h sustained release pattern observed during the *in vitro* dissolution studies. The blunted C_{max} (0.79±0.49 μm) and prolonged t_{max} (3.30±1.20 h) is consistent with the slower drug release compared to the other formulations observed *in vitro*. The lower $AUC_{0\text{-}24}$ from this extended release formulation may be the result of reduced absorption of the drug in the colon after transit from the upper intestine.

A fast absorption (t_{max}=1.00±0.00 h) and significantly higher plasma concentrations were observed in rats treated with torcetrapib-loaded SLNs, resulting in approximately 11.6-fold increases in $AUC_{0\text{-}24}$, slightly better than that of torcetrapib-polymer dispersion. One key factor in enhancing the drug oral bioperformance is the significantly reduced particle size that tremendously increases the surface area for fast drug dissolution, reducing the t_{max}. This is consistent with the fast rate of drug release observed in the *in vitro* dissolution studies.

Based on the *in vitro* dissolution studies, the likely major reason for the increase in the drug oral bioavailability for solid lipid formulations in our study can be ascribed to the increase in the apparent solubility of the drug in the GI tract. Since the drug is dispersed in lipid matrix in amorphous state, it is more soluble than its crystalline counterpart. Moreover, as revealed from the *in vitro* supersaturation studies, the drug released from the SLMs was maintained at a level of supersaturation similar to that from the polymer dispersion. Another possible solubilization mechanism would be the simulation of "food effect". It is well known that high fat meals can significantly enhance the oral absorption of poorly water-soluble drugs by promoting the biliary secretion of bile salts and phospholipids that aid in the solubilization of drug. The presence of exogenous lipids in the GI tract, being similar to the composition of fat meal, could stimulate the biliary secretions as well, leading to the formation of submicron mixed micelles in which the lipophilic molecule is solubilized [4].

CONCLUSION

In this study, we have successfully demonstrated a simple and convenient way of producing solid lipid dispersions loaded with the highly lipophilic drug, torcetrapib. This formulation approach can be performed on a small scale, making it ideal for use in the early drug discovery space when material may be limited. Solid lipid microparticles were prepared via anti-solvent precipitation and subsequently used to create solid lipid nanoparticles by acoustic milling. Both solid lipid micro- and nanoparticles were fully characterized and displayed satisfactory physicochemical properties. Amorphous dispersions of torcetrapib in the lipid matrix were obtained and sustained *in vitro* supersaturation of torcetrapib could be achieved using solid lipid as a vehicle. Uniquely, we have also demonstrated the ability to modulate the drug release rates from solid lipid matrix simply by controlling the precipitation temperature during the formation of the drug-lipid particles as well particle size. *In vivo* pharmacokinetic studies revealed significant boosts in oral bioavailability (~6 to 11-fold) from both solid lipid micro- and nanoparticles relative to the drug powders, comparable to or better than the performance of spray dried amorphous polymer dispersions. These results suggest that solid lipid dispersions are a promising oral delivery system for enhancing and controlling the oral absorption of poorly water-soluble drugs such as torcetrapib.

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