European Journal of Pharmaceutics and Biopharmaceutics 142 (2019) 258–264

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Fenofibrate oral absorption from SNEDDS and super-SNEDDS is not significantly affected by lipase inhibition in rats

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ARTICLE INFO

Keywords:
Absorption
Digestion
Fenofibrate
Orlistat
Supersaturation
Super-SNEDDS
SNEDDS

ABSTRACT

The effect of drug load and digestion on the solubilization and absorption of fenofibrate in self-nanoemulsifying drug delivery system (SNEDDS) was assessed in a pharmacokinetic study in rats and in an *in vitro* lipolysis model. SNEDDS containing fenofibrate at 75% of equilibrium solubility (S_{eq}), a super-saturated SNEDDS (super-SNEDDS) containing fenofibrate at 150% of S_{eq} and a super-SNEDDS suspension containing fenofibrate at 100% of S_{eq} and an additional 50% S_{eq} fenofibrate suspended (150% of S_{eq} in total) were used. To assess the effect of lipid digestion on fenofibrate absorption in rats and fenofibrate solubilization during *in vitro* lipolysis, the lipase inhibitor orlistat was added at 1% (w/w) to the SNEDDS, resulting in six different SNEDDS: SNEDDS, super-SNEDDS and super-SNEDDS suspension with and without orlistat 1% (w/w). *In vivo*, super-SNEDDS had a higher C_{max} and $AUC_{0\text{-}30h}$ compared to SNEDDS and super-SNEDDS suspension, both with and without orlistat. While orlistat did not affect fenofibrate absorption in SNEDDS and super-SNEDDS, an increase of T_{max} and $AUC_{0\text{-}30h}$ for super-SNEDDS suspension was found when orlistat was present. During *in vitro* lipolysis, the addition of orlistat decreased digestion and lowered drug precipitation. Super-SNEDDS showed significantly increased absorption in rats compared to SNEDDS and super-SNEDDS suspension and the inhibition of digestion resulted in prolonged and increased absorption for the super-SNEDDS suspension.

1. Introduction

Administration of drugs via the oral route is often preferred over other routes of administration, due to the ease of manufacturing of oral dosage forms and high patient compliance. However, with an increasing number of poorly water soluble drugs, oral drug development faces significant challenges. Drugs belonging to class II of the biopharmaceutics classification system (BSC) have poor aqueous solubility or low dissolution rates which often lead to a poor and variable absorption resulting in an inconsistent bioavailability {Stegemann, 2007 #92} [1]. Poor aqueous solubility and low dissolution rates, however, can be overcome with drug formulation using advanced “enabling” drug delivery systems [2]. Lipid based drug delivery systems, such as self-nanoemulsifying drug delivery systems (SNEDDS), belong to this type of formulations and have the advantage that the drug is dissolved in the SNEDDS preconcentrate (undispersed, water-free SNEDDS) and thus is administered in solution. Hence, the dissolution step, required when the drug is administered in a crystalline form, is avoided. SNEDDS are isotropic mixtures of oils, surfactants and co-solvents that form nanoemulsions upon gentle agitation in an aqueous environment, such as in the gastro-intestinal tract, and they are typically administered in soft gelatin capsules [3]. Administration of BCS class II drugs in SNEDDS thus typically results in increased bioavailability compared to conventional formulations [4].

In supersaturated SNEDDS (super-SNEDDS), the drug is in solution (supersaturated) in the SNEDDS preconcentrate above its equilibrium solubility (S_{eq}), which increases the thermodynamic activity of the drug [5,6]. Super-SNEDDS provide an alternative to conventional SNEDDS, where the dose of a drug to be administered is limited by the solubility of the drug in the formulation, and is often between 50% and 90% of S_{eq} in the SNEDDS preconcentrate [7–9]. This can result in the need for administration of several SNEDDS containing capsules in order to administer the required dose. In a series of studies by Thomas *et al.*, super-SNEDDS have been found to be superior compared to SNEDDS yielding

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https://doi.org/10.1016/j.ejpb.2019.07.002
Received 28 January 2019; Received in revised form 1 July 2019; Accepted 1 July 2019
Available online 02 July 2019
0939-6411/

the same or higher bioavailabilities for halofantrine, simvastatin, and fenofibrate compared to SNEDDS in mini pigs and dogs [3,10,11]. In agreement with this, in a rat study by Michaelsen *et al*., super-SNEDDS with halofantrine also yielded a higher bioavailability compared to SNEDDS - with the same drug dose administere d, but only half the formulation volume [12]. Correspondingly, Siqueira *et al*. dosed 20 mg/kg of the model drug R3040 as super-SNEDDS (drug load of 200% S_{eq}) and as conventional SNEDDS (drug load of 80% S_{eq}) to rats. Similar drug absorption from both SNEDDS was found, even though the dose of super-SNEDDS contained 2.5 fold less SNEDDS preconcentrates than the conventional SNEDDS [9].

Since SNEDDS are, at least in part, composed of digestible excipients, digestion is thought to be one of the key factors affecting the bioavailability of a drug when dosed in SNEDDS. Upon digestion of the digestible components in a SNEDDS, a variety of colloidal structures with varying solubilizing capacity for the drug are formed [13–16]. The current hypothesis regarding absorption from SNEDDS revolves around the formation of mixed colloidal species, such as vesicles and micelles that enhance drug solubilization in the gastro-intestinal tract and hence decrease the risk of drug precipitation, although it should be noted, that absorption may still be substantial even when significant precipitation is present [10,17]. The drug solubilized in the colloidal structures is in equilibrium with the free drug, which gives an absorption advantage compared to traditional dissolution from a conventional solid oral dosage form.

Orlistat (tetrahydrolipstatin) is known as a potent inhibitor of pancreatic lipase, gastric lipase, and carboxyl ester lipase and has been studied extensively throughout the last decades [18–20]. Orlistat is known to inhibit triglyceride digestion when dissolved in the triglycerides and is thought to inhibit the lipase at a 1:1 M ratio [21]. In the present study orlistat was applied as a tool to inhibit the digestion of the lipids present in the SNEDDS in order to evaluate the effect of lipid digestion on drug absorption. In a previous study where orlistat was co-dosed with halofantrine in SNEDDS, it was found that the extent of absorption of halofantrine was not affected. However, C_{max} decreased (506 ± 112 ng/mL for SNEDDS and 295 ± 53 ng/mL for SNEDDS with orlistat) and the absorption phase was extended (t_{max} of 2.8 ± 1.2 h for SNEDDS and 6.3 ± 1.2 h for SNEDDS with orlistat) [12]. The importance of digestion in absorption from SNEDDS was questioned and an alternative hypothesis regarding absorption from SNEDDS was proposed. When digestion is inhibited, the colloidal structures formed from the digestion products will not be generated and the lipid emulsion droplets will more or less stay intact. Absorption may therefore be facilitated by diffusion from the lipid droplets acting as a reservoir. In addition, the amorphous nature of the halofantrine precipitate studied during dynamic *in vitro* lipolysis was speculated to play a positive role with regard to absorption [12]. In another study by de Smidt *et al*. also using orlistat to investigate the effect of digestion on absorption of penclomidine, similar findings were reported when using emulsified lipid based formulations [22].

The aim of this study was two-fold: first, to evaluate if the drug load, and the state of the drug (dissolved or suspended) in SNEDDS influenced the pharmacokinetics of fenofibrate after oral dosing to rats and secondly, to investigate whether digestion plays a role in the absorption of fenofibrate from SNEDDS, super-SNEDDS and super-SNEDDS suspension.

2. Materials and methods

2.1. Materials

Fenofibrate, fenofibric acid, clofibric acid, soybean oil (long-chain (LC) glycerides), 4-bromophenyl-boronic acid (BBBA), bile extract, tris-(hydroxymethyl)aminomethane (tris), maleic acid, calcium chloride, sodium hydroxide, and porcine pancreatic lipase were obtained from Sigma-Aldrich (St. Louis, MO, USA). Maisine 35–1 (a mixture of LC mono-, di-, and triglycerides) was kindly donated by Gattefossé (St. Priest, France), and Kolliphor RH 40 was donated by BASF (Ludwigshafen, Germany). Orlistat was purchased from Molekula (Newcastle Upon Tyne, UK). Purified water was obtained from a Millipore Milli-Q Ultra Pure water purification system (Billerica, MA, USA). All other chemicals were of analytical grade and were used as received unless specified otherwise.

2.2. Preparation of formulations

SNEDDS preconcentrates were prepared as previously described [11,12,23]. The composition of the SNEDDS was soybean oil (27.5% w/w), Maisine 35–1 (27.5% w/w), Kolliphor RH40 (35% w/w) and absolute ethanol (10% w/w). Briefly, molten Maisine 35–1 was mixed with soybean oil (1:1 w/w). Molten Kolliphor RH40 was then added to the lipids and mixed until homogenous. After cooling to ambient temperature, ethanol was added. The preconcentrates were further stirred until they appeared visually homogenous. Drug loaded SNEDDS were prepared by adding fenofibrate into glass vials and subsequently adding the preconcentrate. The equilibrium solubility of fenofibrate was previously determined by Thomas *et al.* to be 88.5 mg/g and drug load is expressed as percent of S_{eq} [10]. The SNEDDS contained 75% fenofibrate compared to S_{eq} and the super-SNEDDS and super-SNEDDS suspension contained 150% fenofibrate compared to S_{eq}. The SNEDDS were stirred with a magnetic stirring bar at room temperature to aid the drug dissolution process. The super-SNEDDS were ultrasonicated for 30 min and then heated to 60 °C for 3 h and left overnight to cool at 37 °C. Super-SNEDDS suspension contained 100% fenofibrate compared to S_{eq} in solution and an extra 50% compared to S_{eq} added as a solid and was prepared by adding crystalline drug to the SNEDDS preconcentrate. It should thus be noted that a super-SNEDDS suspension does contain a similar total drug concentration as the corresponding super-SNEDDS, but does not contain dissolved drug in a supersaturated state. Complete dissolution of the drug in the super-SNEDDS preconcentrates was confirmed by polarized light microscopy (PLM). Orlistat was used in a final concentration of 1% (w/w) and dissolved in the preconcentrates at room temperature upon stirring. In summary, SNEDDS, super-SNEDDS and super-SNEDDS suspension with and without orlistat at 1% (w/w) led to six formulations, which were used in this study.

2.3. Dynamic in vitro lipolysis

Dynamic *in vitro* lipolysis was carried out under fasted state conditions as previously described [12,23–25]. The SNEDDS were weighed into a thermostated vessel containing 25 mL intestinal lipolysis medium (2.5 mM bovine bile salt, 0.26 mM phospholipid, 2 mM Tris buffer, 2 mM maleic acid and 50 mM sodium chloride). Lipolysis was initiated by the addition of 5 mL freshly prepared pancreatic lipase to the intestinal lipolysis medium leading to a final activity of 550 USP/mL. Pancreatic lipase solution was prepared by weighing the crude porcine pancreatic lipase into a polypropylene tube and adding Milli-Q water. The mixture was vortexed until homogenous and then centrifuged (7 min, 6500 × g, 37 °C). The *in vitro* lipolysis was controlled by continuous addition of calcium (0.5 M; 0.01 mL/min of a solution with $CaCl_2$) throughout the 60 min of *in vitro* lipolysis. The released free fatty acids were continuously titrated with NaOH (0.4 M) by an automated pH-stat (Metrohm Titrino 744, Tiamo Version 1.3, Switzerland) to keep the pH constant at 6.5. Samples (1 mL) were withdrawn from the thermostated vessel at the time points 0, 15, 30, 45 and 60 min. Lipase activity was immediately inhibited by the addition of 5 μL 4-BBBA (1 M in methanol) followed by centrifugation (18,255 × g, 30 min at room temperature). This resulted in separation of the clear supernatant from the pellet. The supernatant was decanted and the pellet isolated with a spatula. Drug content in both phases was quantified by HPLC following appropriate dilution with methanol (see below). The solid state properties of fenofibrate in the pellet was analyzed by X-ray powder

diffraction (XRPD) and PLM after 60 min lipolysis (see below).

2.4. Quantification of lipolysis samples

The amount of fenofibrate in the aqueous and pellet phases after centrifugation and dilution was determined by isocratic high-performance liquid chromatography (HPLC) using a Phenomenex Kinetex 5u C18 100A column (100 × 4.6 mm) protected by a Phenomenex guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 85% (v/v) methanol and 15% (v/v) purified water, the flow rate was 0.9 mL/min, and the injection volume was 20 µL. The detection wavelength was 288 nm, and the drug retention time was approximately 2.4 min. All samples were analyzed at room temperature using a Dionex ASI-100 automated sample injector, P680 HPLC pump, and PDA-100 photodiode array detector (Thermo Fisher Scientific, Waltham, MA, USA). The chromatograms were evaluated using a Thermo Scientific Dionex Chromeleon 7 Chromatography Data System (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. X-ray powder diffraction

The solid state properties of the pellets were analyzed after 60 min of lipolysis by XRPD using an ÝPert Pro X-ray diffractometer (MPD PW3040/60 XRD, PANalytical, Almelo, The Netherlands). The samples were scanned from 5°-35°2θ with a step size of 0.02608°2θ and a speed of 0.03368°/s. As a radiation source CuKα radiation (l.542 Å) was used. The system was operated at a voltage of 40 kV and a current of 30 mA. Each measurement lasted approximately 10 min. The data was collected using X'Pert data collector version 2.2 and analyzed with X'Pert high-score plus software version 2.2.4 (both from PANalaytical B.V.). Control experiments were carried out using pellets from lipolysis of drug-free SNEDDS. The pellets were isolated and spiked with fenofibrate in amounts corresponding to the amounts found in the pellets of super-SNEDDS without orlistat (approx. 13 mg).

2.6. Polarized light microscopy

Microscopic analysis of the pellet was conducted using a Zeiss Axiolab microscope (Carl Zeiss, Göttingen, Germany). The pellets were observed under cross polarizers. Polarized light micrographs were obtained by using a Moticam 10.0 MP digital camera and Motic Images Plus 2.0 software (Hong Kong, China). SNEDDS and super-SNEDDS were observed at a magnification of 40× and 5×, respectively.

2.7. In vivo study

The protocols used in this study were approved by the animal welfare committee appointed by the Danish ministry of justice and conform to the NIH guidelines on animal welfare. The study was carried out in compliance with EC directive 86/609/EEC and with the Danish law regulating animal experiments. Male Sprague Dawley rats (268–290 g) obtained from Harlan (Horst, The Netherlands) were divided randomly into 6 groups of 6 animals each. The animals were allowed to acclimatize for 7 days prior to the experiments and were kept on a standard feed with free access to water. Prior to the study the rats were fasted for 6 h. The animals had free access to water during the experiment and were allowed food 4 h after initiation of the experiment. All animals received 8 mg/kg fenofibrate by oral gavage. All formulations were pre-emulsified with Milli-Q water before dosing (10% (w/w) formulation). The super-SNEDDS were prepared the day before the *in vivo* experiments and were pre-emulsified right before dosing. After pre-emulsification, visual inspection was performed and no sign of fenofibrate precipitation was observed. Blood samples were withdrawn from the tail vein at 30 min and 1, 2, 3, 4, 6, 8, 10, 24 and 30 h after administration and collected into 0.5 mL EDTA coated tubes. Plasma was separated from the samples immediately by centrifugation at (15 min, 4 °C, 1651*g*) and stored at −80 °C until analysis. The rats were euthanized 30 h after dosing.

2.8. Bioanalysis

The preparation of plasma samples was based on a method modified from Berthelsen *et al.* [26]. This method is originally adapted from Hanafy *et al.* [27]. The process was carried out as follows; 50 µL plasma was mixed with 20 µL internal standard (clofibric acid in methanol, 200 µg/mL) followed by addition of 100 µL acetonitrile. The mixture was briefly vortexed and subsequently ultrasonicated in an ultrasonic water bath for 10 min. The samples were stored at − 20 °C for 10 min, followed by centrifugation (14 min, 20,227 × g) at 0 °C. The clear supernatant was transferred into HPLC vials and analyzed by HPLC. The samples for the standard calibration curve were prepared similar to the plasma samples with spiked blank plasma. The standard curves were run in the concentration range 0.06–20.0 µg/mL and were linear over the entire range. The concentration of fenofibric acid in the plasma samples was determined by isocratic HPLC using a Phenomenex Kinetex 5u XB-C18 100A column (100 × 4.6 mm) (Phenomenex, Torrance, CA, USA) and a Dionex ASI-100 automated sample injector, P680 HPLC pump, and PDA-100 photodiode array detector (Thermo Fisher Scientific, Waltham, MA, USA). The chromatograms were evaluated using the Thermo Scientific Dionex Chromeleon 7 Chromatography Data System (Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of formic acid 0.1% (v/v): methanol (32:68 v/v). The flow rate was 0.6 mL/min and the injection volume was 20 µL. Fenofibric- and clofibric acid were measured at 287 nm with approximate retention times of 8 and 4 min, respectively.

2.9. Pharmacokinetic analysis

All pharmacokinetic parameters were calculated using WinNonlin Professional Version 6.3 (Pharsight Coporation, Mountainview, CA, USA). The area under the curve (AUC) was determined using the linear trapezoidal model from t = 0 to t = 30 h. $AUC_{0\text{-}30h}$, maximum plasma concentration (C_{max}), time to reach the maximum plasma concentration (t_{max}), and the half-life ($t_{½}$) were determined from the individual plasma curves. All data was normalized to dose.

2.10. Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.03 (GraphPad Software, San Diego, CA, USA). Data is expressed as mean ± standard error of the mean (SEM) and the data was analyzed using ANOVA followed by Šídák's multiple comparison test ($\alpha = 0.05$).

3. Results

3.1. In vivo study

The plasma concentration curves of fenofibric acid are depicted in Fig. 1. Fenofibrate was dosed at 8 mg/kg body weight resulting in 34.3 mg lipid/kg for the SNEDDS and 17.2 mg lipid/kg for the super-SNEDDS and super-SNEDDS suspension.

A noncompartmental model was used to estimate the pharmacokinetic parameters including C_{max}, t_{max}, $t_{½}$ and $AUC_{0\text{-}30h}$ of fenofibrate dosed in SNEDDS, super-SNEDDS and super-SNEDDS suspension with and without orlistat (Table 1). C_{max} and $AUC_{0\text{-}30h}$ for the super-SNEDDS (13.3 ± 4.7 µg/mL and 148.0 ± 47.5 h*µg/mL respectively) are significantly higher than for the SNEDDS (7.4 ± 1.7 µg/mL and 88.3 ± 20.9 h*µg/mL respectively) ($p < 0.05$). As no significant differences in C_{max}, $AUC_{0\text{-}30h}$, and t_{max} are observed when comparing SNEDDS and super-SNEDDS to the SNEDDS and super-SNEDDS containing orlistat, it is suggested that orlistat had not effect in either extent or rate of absorption for SNEDDS and super-SNEDDS (Fig. 1 a and

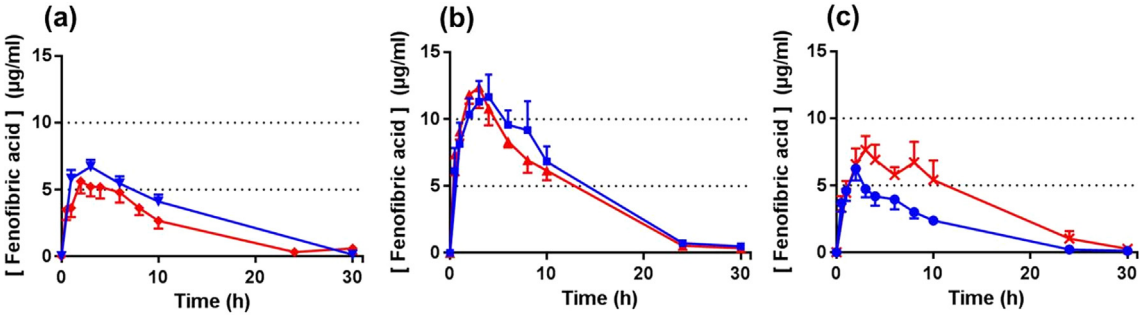

Fig. 1. Plasma concentration of fenofibric acid following oral administration of (a) SNEDDS (b) super-SNEDDS and (c) super- SNEDDS suspension in rats. The treatments included SNEDDS (▼), SNEDDS with orlistat (♦), super-SNEDDS (■), super-SNEDDS with orlistat (▲), super-SNEDDS suspension (●) and super-SNEDDS suspension with orlistat (x). Data represents mean ± SEM, n = 6.

b). For the super-SNEDDS suspension with and without orlistat, there is a significant change in t_{max} ($p < 0.05$) and therefore, the addition of orlistat prolongs the absorption phase. Furthermore, the $AUC_{0\text{-}30h}$ is significantly larger for the super-SNEDDS suspension with orlistat (108.9 ± 39.5 h*µg/mL) than for the super-SNEDDS suspension without orlistat (58.1 ± 16.9 h*µg/mL).

3.2. Dynamic in vitro lipolysis

SNEDDS, super-SNEDDS and super-SNEDDS suspensions were subjected to dynamic *in vitro* lipolysis in order to determine the degree of digestion and to evaluate the capacity of the SNEDDS to solubilize fenofibrate throughout the 60 min lipolysis. Fig. 2 shows that the total amount of released fatty acids was higher for SNEDDS than for super-SNEDDS and super-SNEDDS suspension, which is related to the higher amount of SNEDDS added to the system, compared to the super-SNEDDS. The addition of orlistat decreased the released fatty acids to similar values for SNEDDS, super-SNEDDS and super-SNEDDS suspension (approx. 3%).

Fig. 3 illustrates the relative distribution of fenofibrate in the aqueous phase *(grey)* and the pellet phase *(black)*. During the course of lipolysis, precipitation of fenofibrate is constant for SNEDDS (Fig. 3a) and increases gradually for super-SNEDDS (Fig. 3b) and for super-SNEDDS suspension (Fig. 3c). For the SNEDDS and super-SNEDDS with orlistat (Fig. 3d and e), the precipitation remains constant throughout lipolysis and there is no significant difference when comparing 0 min and 60 min ($p > 0.05$). For super-SNEDDS suspension (Fig. 3f), however, an increase in precipitation from 0 min to 60 min was observed ($p < 0.05$).

3.3. Solid state characterization of pellets

The solid state properties of the pellets after dynamic *in vitro* lipolysis were investigated by XRPD and PLM. Pure fenofibrate corresponding to the crystalline form I of the drug (Fig. 4a) exhibited several characteristic reflections between 13 and 32°2θ with the most pronounced reflections found at approximately 21–22°2θ [28]. The characteristic diffraction pattern of fenofibrate form I was also visible in the diffractograms of the pellets from the super-SNEDDS (Fig. 4c) and the

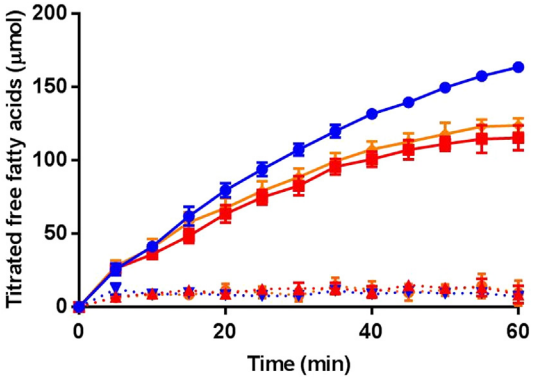

Fig. 2. Titrated free fatty acids from hydrolysis of SNEDDS (●), super-SNEDDS (■), super-SNEDDS suspension (♦), SNEDDS with orlistat (..▼..), super-SNEDDS with orlistat (..▲..) and super-SNEDDS suspension with orlistat (..●..) during 60 min dynamic *in vitro* lipolysis. Data represents mean ± SEM, n = 3.

spiked sample (Fig. 4g). In the pellets from the SNEDDS, the SNEDDS with orlistat and the super-SNEDDS with orlistat, no crystalline fenofibrate was detected by XRPD. To further evaluate the presence of crystalline fenofibrate, PLM was utilized and could confirm the presence of crystalline precipitate in both the SNEDDS and the super-SNEDDS without orlistat. The crystals appeared, however, smaller and more needlelike and elongated for the super-SNEDDS than for the SNEDDS upon inspection of the PLM micrographs (Fig. 4b and c). No precipitation was observed for the SNEDDS and super-SNEDDS containing orlistat.

4. Discussion

An increased absorption when dosing poorly water soluble drugs in lipid based drug delivery systems such as SNEDDS is well documented and super-SNEDDS have proven to provide a good strategy to increase drug absorption even further from SNEDDS [3,10,23]. Digestion has been speculated to play a critical role for absorption of these types of systems. However, the exact mechanism of the observed increase in drug absorption for SNEDDS, super-SNEDDS and super-SNEDDS

Table 1
Pharmacokinetic parameters of fenofibric acid following oral administration of fenofibrate to male Sprague Dawley rats in SNEDDS, super-SNEDDS and super-SNEDDS suspension with and without orlistat.

	SNEDDS	SNEDDS w. orlistat	Super-SNEDDS	Super-SNEDDS w. orlistat	Super-SNEDDS suspension	Super-SNEDDS suspension w. orlistat
C_{max} (µg/mL)	7.4 ± 1.7[a]	6.5 ± 1.1	13.3 ± 4.7[a,b]	13.7 ± 3.1	6.6 ± 1.8[b]	9.4 ± 3.1
T_{max} (h)	2.3 ± 1.4	2.9 ± 1.9	4.3 ± 2.3	2.7 ± 0.8	2.1 ± 0.9[c]	4.7 ± 2.0[c]
t½ (h)	4.2 ± 0.6	5.0 ± 1.2	5.1 ± 1.2	4.8 ± 0.3	4.5 ± 0.6	4.0 ± 2.0
$AUC_{0\text{-}30h}$ (h*µg/mL)	88.3 ± 20.9[a]	66.3 ± 14.9	148.0 ± 47.5[a, b]	136.9 ± 27.5	58.1 ± 16.9[b,c]	108.9 ± 39.5[c]

Data represents mean ± SEM, n = 6. Values with the same letters indicate significant differences (ANOVA with Šídák's multiple comparison test with α 0.05).

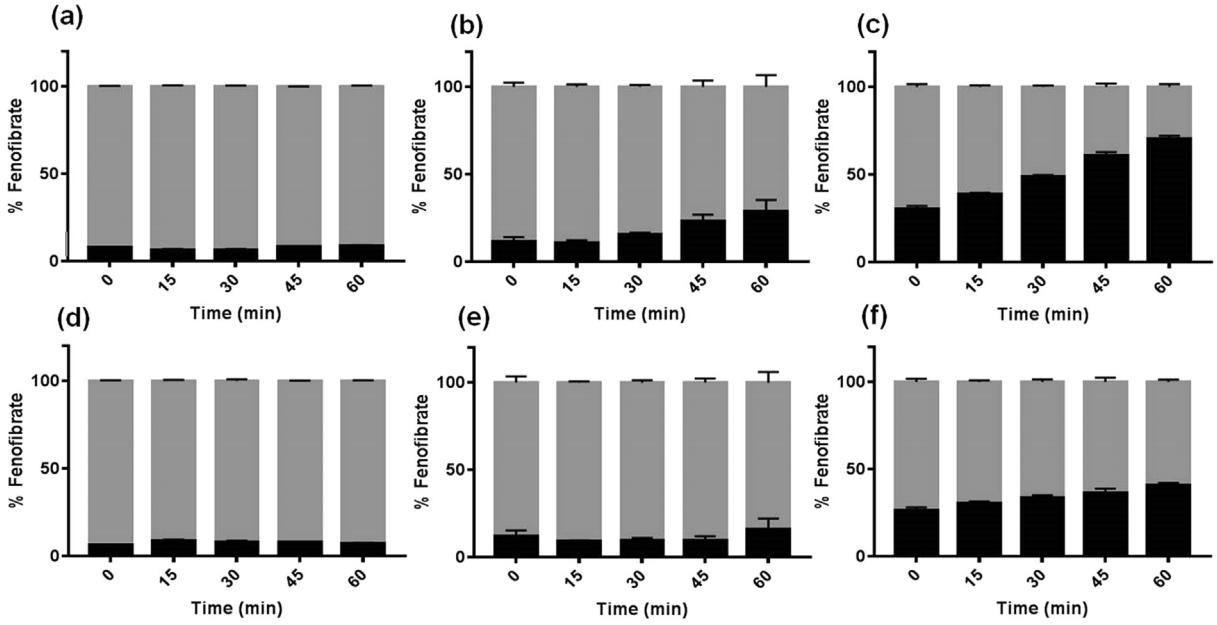

Fig. 3. Relative amount of fenofibrate in the pellet (*black)* and the aqueous phase *(grey)* during lipolysis for SNEDDS (a), super-SNEDDS (b), super-SNEDDS suspension (c), SNEDDS with orlistat (d), super-SNEDDS with orlistat (e) and super-SNEDDS suspension with orlistat (f). Data represents mean ± SEM, n = 3.

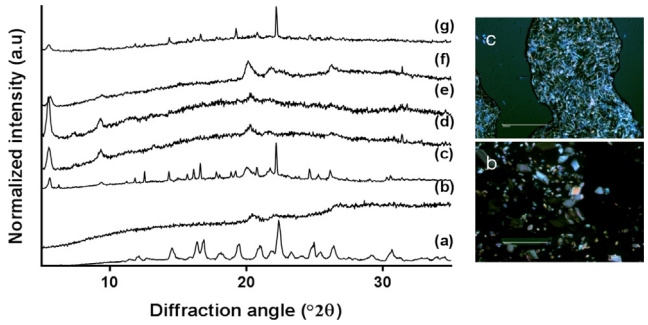

Fig. 4. XRPD diffractograms and polarized light micrographs of crystalline fenofibrate (a), pellets obtained from SNEDDS (b), pellets obtained from super-SNEDDS (c), pellets obtained from SNEDDS with orlistat (d), pellets obtained from super-SNEDDS with orlistat (e), pellets obtained from blank SNEDDS (f) and blank pellets spiked with fenofibrate (approx. 13 mg) (g). All samples were taken after 60 min *in vitro* lipolysis and centrifugation.

suspension is still unknown.

The current study set out to investigate the effect of drug load in the formulations and digestion on absorption of the poorly water soluble drug fenofibrate. Digestion of triglycerides and diglycerides by pancreatic lipase releases digestion products, *e.g.* monoglycerides and fatty acids, which together with bile salts and phospholipids provide high drug solubilization capacity and may enhance drug absorption [29]. Therefore, orlistat was used in this study as a lipase inhibitor to further investigate the role of lipid digestion on drug absorption in a pharmacokinetic study in rats and drug solubilization during *in vitro* lipolysis. In a previous study, in which the amorphous precipitating drug halofantrine was dosed to rats in SNEDDS and super-SNEDDS in the presence or absence of orlistat, it was speculated that the reason for the observed effects *in vivo* were, at least in part, due to the amorphous precipitate formed during *in vitro* lipolysis [12]. Fenofibrate was therefore chosen as the model drug, as it precipitates crystalline during *in vitro* lipolysis [10].

4.1. In vivo study

Recent studies using super-SNEDDS as a novel drug delivery system for poorly water soluble drugs have shown that super-SNEDDS and super-SNEDDS suspension give rise to an equal or higher bioavailability than SNEDDS [3,10,23]. These studies have been performed in dogs and minipigs and more recently, super-SNEDDS have been studied in a rat model [9,12]. In a study conducted by Thomas *et al.,* SNEDDS, super-SNEDDS and super-SNEDDS suspension with fenofibrate were tested in minipigs and all formulations yielded comparable plasma curves despite large differences in the *in vitro* data [10]. From the *in vivo* data in the present study, it is evident that the super-SNEDDS give rise to higher C_{max} and $AUC_{0\text{-}30h}$ compared to SNEDDS, which is in contrast to the aforementioned study by Thomas *et al.* [10]. It is, however, unclear, to which extent the precipitation of fenofibrate dosed in SNEDDs occurs *in vivo* [30]. However, from the *in vitro* studies, the PLM micrographs suggest that the morphology of the precipitates from the SNEDDS and the super-SNEDDS differs. Fenofibrate in the SNEDDS precipitates as large diamond shaped crystals, whereas fenofibrate precipitate from the super-SNEDDS consists of more elongated needle-shaped crystals. This difference in crystal morphology may be important for the interpretation of the *in vivo* studies, if precipitation in fact occurs *in vivo*. Hanafy *et al.* showed in a study with micronized fenofibrate (5 μm in mean particle size) and solid lipid nanoparticles (58 nm in mean particle size) loaded with fenofibrate, that particle size is important for subsequent bioavailability [27]. From the Noyes-Whitney equation it is clear that a reduction in particle size will give an increase in dissolution rate [31]. The smaller fenofibrate crystals seen in the precipitate for the super-SNEDDS may therefore quickly be redissolved and hence play a positive role with regard to the increased C_{max} and AUC.

Digestion is believed to play a major role for absorption of poorly water soluble drugs from SNEDDS [32–34]. However, in the present study, no significant differences are seen when dosing fenofibrate in SNEDDS or super-SNEDDS with or without orlistat. In a recent study by the authors, inhibiting digestion of SNEDDS loaded with halofantrine, it was also found that digestion did not play a role for the AUC, however, inhibition of digestion delayed the t_{max}, and thus changes the plasma profile [12]. The colloidal structures formed upon digestion have been thought to play a major role with regard to the trafficking of drug from the lumen to the absorptive membrane; however, the present data indicates that fenofibrate have a higher propensity to partition out of the undigested nano-emulsion droplets and into the mixed bile salt

micelles, that will still be present in the intestine. In a study by Yeap *et al.*, cinnarizine absorption from a model colloidal system containing long chain fatty acids was investigated using amiloride as a fatty acid absorption inhibitor in a perfusion set-up in rats [35]. They found that in the presence of amiloride, cinnarizine absorption decreased significantly and thus concluded that formulations containing absorbable lipids would be more effective in enhancing absorption of poorly water soluble drugs. In contrast, de Smidt *et al.* showed that digestion did not play a critical role for absorption of penclomidine from emulsions containing medium chain lipids co-dosed with orlistat [22].

The super-SNEDDS suspension is the only formulation in the present study where there is an effect of digestion. T_{max} and $AUC_{0\text{-}30h}$ are significantly increased for the super-SNEDDS suspension with orlistat compared to the super-SNEDDS suspension without orlistat. When digestion is inhibited, the nanoemulsion droplets will continue to exist throughout the gastrointestinal transit and thus provide a constant dissolution compartment for the drug. Partitioning of fenofibrate from the nanoemulsion droplets to the endogenous colloidal structures such as bile salt micelles will thus facilitate absorption.

4.2. In vitro lipolysis

In vitro lipolysis is frequently used in development of lipid formulations, such as SNEDDS, in order to evaluate the degree of digestibility and the ability of the digested SNEDDS to keep the drug solubilized [36]. One common assumption is that if the formulation can keep the drug solubilized during the digestion process, a higher bioavailability may be achieved [37]. During lipolysis, the lipid components of the SNEDDS will be digested, resulting in the formation of various and transient colloidal structures composed of digestion products and components of the biorelevant medium (bile salts and phospholipids). Since the dose of fenofibrate was kept constant in these experiments, the amount of lipids present in the super-SNEDDS and super-SNEDDS suspension is only half of that of the SNEDDS, which resulted in higher fenofibrate precipitation for super-SNEDDS and super-SNEDDS suspension in the absence of orlistat (Fig. 3 a, b and c).

Orlistat is known as a potent lipase inhibitor of gastric, pancreatic and carboxyl ester lipase [19]. In this work, orlistat significantly inhibited digestion ($p < 0.05$) (Fig. 2) and the level of precipitated fenofibrate was practically constant throughout *in vitro* lipolysis (Fig. 3 d, e and f). The lower level of precipitation, as compared to the formulations without orlistat, is most likely related to the inhibition of lipase activity (Fig. 2), which retained the solubilization capacity of SNEDDS, super-SNEDDS and super-SNEDDS suspension. The apparent higher fenofibrate precipitation in super-SNEDDS suspension (Fig. 3 c and f), compared to fenofibrate precipitation from super-SNEDDS (Fig. 3 b and e), is related to the original crystals of fenofibrate in the super-SNEDDS suspension, which were not dissolved during *in vitro* lipolysis.

After 60 min of *in vitro* lipolysis, 8.7 ± 0.9% of the drug dose has precipitated from the SNEDDS, whereas 28.7 ± 6.7% has precipitated from super-SNEDDS and 70.5 ± 1.7% of the fenofibrate dose are found in the pellet from super-SNEDDS suspension. The higher solubilization capacity of super-SNEDDS compared to super-SNEDDS suspension is probably related to fenofibrate being dissolved in the super-SNEDDS, which produces a similar solubilization profile as for SNEDDS. Thus, the drug load (75% for SNEDDS and 150% for super-SNEDDS) did not have a significant influence on drug solubilization during *in vitro* digestion, when fenofibrate was dissolved in SNEDDS.

4.3. In vivo versus in vitro results

During *in vitro* lipolysis, it is believed that the fraction of drug in the aqueous phase is the fraction available for absorption. Thus, the *in vitro* results from this study suggest that SNEDDS and super-SNEDDS would provide higher absorption than super-SNEDDS suspension (Fig. 3). However, *in vivo*, super-SNEDDS with and without orlistat showed higher absorption than SNEDDS and super-SNEDDS suspension with or without orlistat (Fig. 1). Thus, the *in vitro* lipolysis data could not predict the absorption of fenofibrate in SNEDDS, super-SNEDDS and super-SNEDDS suspension. In this context it has previously been demonstrated by Sassene *et al.,* that fenofibrate loaded in lipid formulations dosed in rats precipitated in the stomach of rats, but not in the intestine [30]. The absence of drug precipitation in the rat intestine suggests that gastric digestion is sufficient to cause drug precipitation. As in this work the *in vitro* lipolysis did not have gastric step, it is possible that the *in vitro* lipolysis model overpredicted fenofibrate precipitation *in vivo*, leading to lack of *in vivo in vitro* correlation.

5. Conclusion

The present study demonstrates that super-SNEDDS loaded with fenofibrate have a superior *in vivo* performance compared to SNEDDS in a rat model. The super-SNEDDS had both a significantly higher C_{max} and AUC compared to the SNEDDS. Thus, the high drug load of super-SNEDDS did not hinder fenofibrate absorption in rats. The improved performance of super-SNEDDS compared to SNEDDS has been shown previously in a range of animal models with different poorly water soluble drugs.

Orlistat did not influence the absorption of fenofibrate from SNEDDS and super-SNEDDS, and therefore, digestion of SNEDDS did not play a significant role with regard to absorption of fenofibrate in rats when the drug was dosed dissolved in SNEDDS. However, when part of the drug was present in suspension, inhibition of digestion with orlistat changed the pharmacokinetic parameters significantly and resulted in a prolonged absorption phase and subsequent increase in t_{max}, C_{max} and AUC. It is proposed that the mechanism behind the increased absorption from super-SNEDDS suspension with orlistat is the dissolution of the drug into the intact nanoemulsion droplets, followed by partitioning of the drug from the nanoemulsion droplets to the endogenous bile salt micelles, which will facilitate absorption, however; further studies are needed in order to understand the underlying mechanism behind drug absorption from SNEDDS and super-SNEDDS.

Acknowledgements

Funding for this project was given by the Canadian Institute of Health Research granted to KMW. A special thanks goes to University of British Columbia, Canada and University of Copenhagen, Denmark, for providing a platform for collaboration.

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