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Investigating the correlation between *in vivo* absorption and *in vitro* release of fenofibrate from lipid matrix particles in biorelevant medium

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ABSTRACT

Lipid matrix particles (LMP) may be used as better carriers for poorly water-soluble drugs than liquid lipid carriers because of reduced drug mobilization in the formulations. However, the digestion process of solid lipid particles and their effect on the absorption of poorly water-soluble drugs are not fully understood. This study aimed at investigating the effect of particle size of LMP on drug release *in vitro* as well as absorption *in vivo* in order to get a better understanding on the effect of degradation of lipid particles on drug solubilisation and absorption. Fenofibrate, a model poorly water-soluble drug, was incorporated into LMP in this study using probe ultrasound sonication. The resultant LMP were characterised in terms of particle size, size distribution, zeta potential, entrapment efficiency, *in vitro* lipolysis and *in vivo* absorption in rat model. LMP of three different particle sizes i.e. approximately 100 nm, 400 nm, and 10 μm (microparticles) were produced with high entrapment efficiencies. The *in vitro* lipolysis study showed that the recovery of fenofibrate in the aqueous phase for 100 nm and 400 nm LMP was significantly higher ($p < 0.05$) than that of microparticles after 30 min of lipolysis, suggesting that nano-sized LMP were digested to a larger extent due to greater specific surface area. The 100 nm LMP showed faster initial digestion followed by 400 nm LMP and microparticles. The area under the plasma concentration–time curve (AUC) following oral administration of 100 nm LMP was significantly higher ($p < 0.01$) than that of microparticles and fenofibrate crystalline suspension (control). However, no significant difference was observed between the AUCs of 100 nm and 400 nm LMP. The same rank order on the *in vivo* absorption and the *in vitro* response was observed. The recovery (%) of fenofibrate partitioning into the aqueous phase during *in vitro* lipolysis and the AUC of plasma concentration–time curve of fenofibric acid was in the order of 100 nm LMP > microparticles > control. In summary, the present study demonstrated the particle size dependence of bioavailability of fenofibrate loaded LMP in rat model which correlates well with the *in vitro* drug release performed in the biorelevant medium.

1. Introduction

Oral drug administration is desirable due to good patient convenience and consequently a better compliance. However, a large number of new chemical entities and many existing drugs are poorly water-soluble (Lipinski, 2000), which poses a challenge for their solubilisation in gastrointestinal fluids when administered orally. Lipid-based drug delivery systems offer a great potential for enhancing the oral bioavailability of lipophilic drugs by rendering them in a solubilised form (Mu et al., 2013). However, drug precipitation during dispersion of liquid lipid-based formulations in the aqueous medium or during lipid digestion processes (Mohsin et al., 2009; Ren et al., 2013) can be a limiting factor for drug absorption. Lipid matrix particles (LMP), such as solid lipid nanoparticles and nanostructured lipid carriers may have advantages in reducing drug mobilisation and preventing drug precipitation during lipid digestion processes in the gastrointestinal tract (GIT)

Abbreviations: ANOVA, analysis of variance; AUC, area under curve; BBBA, 4-bromobenzeneboronic acid; C_{max}, maximal plasma concentration; FFAs, free fatty acids; GIT, gastro-intestinal tract; GMS, glyceryl monostearate; HPLC, high performance liquid chromatography; HPMC, hydroxy propyl methylcellulose; IVIVC, *in vitro–in vivo* correlation; LMP, lipid matrix particles; MCT, medium chain triglyceride; SDS, sodium dodecyl sulfate; SEM, standard error of mean; SNEDDS, self nano-emulsifying drug delivery systems; T_{max}, time required for maximal plasma concentration; USP, United States pharmacopeia.

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and, hence, be used to increase the oral bioavailability (Hu et al., 2004; Müller et al., 2008; Souto and Müller, 2010).

LMP can consist of solid lipids or a combination of solid and liquid lipids (Muchow et al., 2008; Müller et al., 2000; Westesen et al., 1997). The improvement of drug solubilisation in the GIT using LMP depends on lipid excipients and lipid digestion process, because lipid digestion products of the digestible lipids play an important role in micellar formation (Carey et al., 1983; Cuiné et al., 2008). It has been shown that apparent solubility of poorly water-soluble drug, which accounts for both solubilised drug in molecular state and by means of micelles, increased in simulated intestinal medium due to the formation of micelles (Buckley et al., 2013; Frank et al., 2012). Our hypothesis is that the drug is slowly released from LMP upon the enzymatic hydrolysis of the lipid matrix. The endogenously secreted bile mixture (bile salts, phospholipids, cholesterol) and products of lipid hydrolysis (free fatty acids (FFAs), monoglycerides, and diglycerides) assemble themselves to form submicron colloidal structures, e.g. micelle, which may prevent precipitation of drug compound by accommodating them into the micelles (Dahan and Hoffman, 2008; Holm et al., 2013; Porter et al., 2007).

The dynamic *in vitro* lipolysis has shown advantages in assessing lipid digestion and drug release processes (Dahan and Hoffman, 2008; Zangenberg et al., 2001a; Zangenberg and Kristensen, 2001). Several studies have been carried out involving aqueous dissolution medium for evaluating drug release from various lipid-based drug delivery systems (Chen et al., 2009; Hu et al., 2004; Mu et al., 2013; Xie et al., 2011); however, no research to our knowledge is available evaluating *in vitro* lipid digestion in biorelevant media and *in vivo* response for drug loaded LMP. The present study investigated the effect of particle size of fenofibrate loaded LMP on *in vitro* drug release during lipolysis and *in vivo* absorption in rats, aiming at a better understanding of drug release mechanism of LMP. The knowledge attained from this study can be used in rational design of LMP based formulations in the future.

2. Materials and methods

2.1. Materials

Fenofibrate (99%), clofibric acid (97%), pancreatin from porcine pancreas and 4-bromobenzeneboronic acid (BBBA) (95%) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). The enzyme activity was at least equivalent to 3 × U.S.P. specifications. Fenofibric acid (98%) was purchased from AK Scientific Inc. (Union City, CA, USA). Glyceryl monostearate (GMS, 40–55%), sodium dodecyl sulphate (SDS) and medium-chain triglycerides (MCT) were obtained from Unikem (Copenhagen, Denmark). Poloxamer-407 (Lutrol®F127) was obtained from BASF (Ludwigshafen, Germany). Hydroxy propyl methylcellulose (Metolose 90SJ-4000 SR) was obtained from ShinEtsu (Tokyo, Japan). Sodium chloride (99.5%), chloroform (99.8%), calcium chloride dihydrate (99.0–102.0%), sodium hydroxide pellets, (99%), and formic acid (98–100%) were purchased from Merck (Darmstadt, Germany). Maleic acid (99%) and bovine bile extract were obtained from Fluka Chemi AG (Buchs, Switzerland). Phosphatidylcholine (99%) was purchased from Lipoid (Ludwigshafen, Germany). Methanol (99.8%) was purchased from VWR (Radnor, PA, USA). Acetonitrile (99.9%) was obtained as free samples from Vetec Química Fina (Rio de Janeiro, Brazil).

2.2. Preparation of LMP

Fenofibrate loaded lipid nanoparticles were prepared by the melting-probe sonication method. Solid lipid GMS and liquid lipid MCT were melted along with fenofibrate on a water bath at 75 °C. The melted lipid phase was dispersed into 20 mL of aqueous surfactant solution containing 3% (w/v) Poloxamer-407 and 0.1% (w/v) SDS using magnetic stirring at 75 °C to form an oil in water (O/W) pre-emulsion containing 6.25% (w/v) GMS, 3.13% (w/v) MCT and 0.63% (w/v) fenofibrate. The O/W pre-emulsion was subjected to probe sonication with an ultrasonic probe (50/60 kHz, 230 V, Chemical Instruments AB, Sweden) at 80 watts for 10 min to obtain 100 nm LMP and 20 s to obtain 400 nm LMP. The period of ultrasound burst was set to 2 s with a pause of 2 s between two ultrasound bursts. After probe sonication, the sample was allowed to cool to the room temperature, which resulted in a dispersion containing fenofibrate loaded lipid nanoparticles. The fenofibrate loaded lipid microparticles were prepared as described above with the exception that the magnetically stirred O/W pre-emulsion was not subjected to probe sonication. Fenofibrate micro-crystal suspension (control formulation) was prepared by grinding the raw crystals in a glass mortar which were suspended in 0.7% HPMC solution (5000 cP) containing 5 mg/mL drug.

2.3. Characterisation of LMP

Particle size (given as z-average which is an intensity-based value), size distribution (given as polydispersity index) and zeta potential of fenofibrate loaded lipid particles were determined by the use of Zetasizer from Malvern Instruments (Malvern, UK). The prepared lipid particle dispersions were diluted 25 times using Milli-Q water and whirl mixed before determination. The parameters for Zetasizer were set as follows: scattering angle was 173°, refractive index was 1.33, viscosity was 0.89 cP and the temperature was 25 °C. The particle size was determined as the mean diameter.

The drug entrapment efficiency was determined indirectly by measuring the concentration of the free drug in the aqueous phase of the dispersion using the following equation:

$$\textit{Entrappment Efficiency}\ (\%) = \frac{W_{total} - W_{free}}{W_{total}} * 100$$

The LMPs and the free drug in the suspension (0.5 mL) were separated by ultrafiltration-centrifugation using centrifugal filters (Amicon® Ultra-0.5 with 50 kDa cut-off, Millipore, USA) at 5204*g* for 15 min at the room temperature; the amount of fenofibrate in the ultrafiltrate was regarded as W_{free}. W_{total} was measured by dissolving 0.5 mL of LMP dispersion with 4.5 mL acetone at 50 °C, and the drug concentration in the solution was quantified by HPLC.

The analysis of fenofibrate was performed on Dionex HPLC system (Dionex Corporation, CA, USA) using a C18 hydrosphere column (5 μm, 150 mm × 4.60 mm) with methanol:water (80:20) as the mobile phase and a flow rate of 1.2 mL/min. The UV detection wavelength was 287 nm. The chromatograms were analysed using Chromeleon software (Dionex Corporation, CA, USA).

2.4. Quantification of drug release in vitro

The intestinal *in vitro* lipolysis model developed by Zangenberg et al. (Zangenberg et al., 2001a; Zangenberg and Kristensen, 2001) was used, with some modifications, to investigate the lipid digestion and solubilisation of the drug in the biorelevant media. The biorelevant medium, which was comprised of 32.5 mL of digestion buffer (20 mM maleic acid, 68 mM NaCl, pH 6.5) containing 3 mM bovine bile extract and 0.75 mM phosphatidylcholine, was continuously stirred and maintained at 37 °C. A suspension of LMP (2.5 mL) was then dispersed in the medium and stirred for 5 min. Pancreatic lipase extract was freshly prepared by adding porcine pancreatin extract (320 mg) to 8 mL of the digestion buffer. After stirring, followed by centrifugation (4000 rpm, 7 min at 37 °C) in Heraeus Megafuge 16R centrifuge (Germany), 5 mL of the