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'Stealth' lipid-based formulations: Poly(ethylene glycol)-mediated digestion inhibition improves oral bioavailability of a model poorly water soluble drug

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ABSTRACT

For over 20 years, stealth drug delivery has been synonymous with nanoparticulate formulations and intravenous dosing. The putative determinants of stealth in these applications are the molecular weight and packing density of a hydrophilic polymer (commonly poly(ethylene glycol) (PEG)) that forms a steric barrier at the surface of the nanoparticle. The current study examined the potential translation of the concepts learned from stealth technology after intravenous administration to oral drug delivery and specifically, to enhance drug exposure after administration of oral lipid-based formulations (LBFs) containing medium-chain triglycerides (MCT). MCT LBFs are rapidly digested in the gastrointestinal tract, typically resulting in losses in solubilisation capacity, supersaturation and drug precipitation. Here, non-ionic surfactants containing stealth PEG headgroups were incorporated into MCT LBFs in an attempt to attenuate digestion, reduce precipitation risk and enhance drug exposure. Stealth capabilities were assessed by measuring the degree of digestion inhibition that resulted from steric hindrance of enzyme access to the oil–water interface. Drug-loaded LBFs were assessed for maintenance of solubilising capacity during *in vitro* digestion and evaluated *in vivo* in rats. The data suggest that the structural determinants of stealth LBFs mirror those of parenteral formulations, i.e., the key factors are the molecular weight of the PEG in the surfactant headgroup and the packing density of the PEG chains at the interface. Interestingly, the data also show that the presence of labile ester bonds within a PEGylated surfactant also impact on the stealth properties of LBFs, with digestible surfactants requiring a PEG Mw of ~1800 g/mol and non-digestible ether-based surfactants ~800 g/mol to shield the lipidic cargo. *In vitro* evaluation of drug solubilisation during digestion showed stealth LBFs maintained drug solubilisation at or above 80% of drug load and reduced supersaturation in comparison to digestible counterparts. This trend was also reflected *in vivo*, where the relative bioavailability of drug after administration in two stealth LBFs increased to 120% and 182% in comparison to analogous digestible (non-stealth) formulations. The results of the current study indicate that self-assembled "stealth" LBFs have potential as a novel means of improving LBF performance.

1. Introduction

Drug bioavailability from an oral formulation in the gastrointestinal tract (GIT) is heavily reliant on favourable physiochemical characteristics, including adequate solubility and permeability and resistance to metabolism. However, increasing numbers of new chemical entities (NCE) derived from, e.g., combinatorial and high throughput screening processes, do not meet these criteria [1] and as a result, attrition rates in early stage clinical development are rising [2,3]. Lipid-based drug delivery systems are well established as a means to circumvent the low solubility issues associated with hydrophobic drugs [2,4,5]. The past 20 years have seen lipid-based formulations (LBFs) advance from simple one-excipient or binary systems to more complex multi-component self-emulsifying drug delivery systems (SEDDS) [6–11]. Despite this progressive rise in popularity, however, LBFs occupy less than 4% of the oral market and the development of LBFs remains largely empirical [2].

One limitation to the wider use of medium-chain triglyceride (MCT) containing LBFs is the realisation that these formulations often exhibit a rapid loss in drug solubilisation capacity when subjected to digestion by pancreatic enzymes [12–16]. Under digesting conditions, there is a risk that dissolved drug will precipitate into a more slowly dissolving crystalline form, leading to reduced bioavailability. Judicious design of lipidic formulations is therefore required to generate formulations that are able to withstand the solubilisation challenges encountered on digestion.

Lipid digestion in the GIT is catalysed by the lipase superfamily of interfacially active enzymes [17]. Nonspecific adsorption of the inactive lipase/co-lipase enzyme complex to the surface of an emulsified oil

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droplet results in a conformational change in the enzyme to the active form. The interfacial activation of pancreatic lipase renders the lipolysis reaction highly sensitive to changes at the oil–water interface [17,18]. The presence of commonly used polyethoxylated non-ionic surfactants has previously been reported to modify the rate of *in vitro* lipid digestion [19–24]; however, the available data are contradictory. Thus, increases in lipid digestion rates have been observed on surfactant addition to LBFs, and tentatively attributed to enhanced solubilisation of digestion products [19], while reduced digestion has also been reported and attributed to steric hindrance of lipase access to the oil–water interface [21–24].

PEGylated (stealth) drug delivery vehicles comprising a polyethoxylated steric stabilisation layer have been extensively utilised in parenteral drug delivery to enhance plasma circulation times and to promote drug accumulation at sites of hypervascularisation such as tumours or inflamed tissues [25–27]. The stealth effect attributed to these drug delivery systems can be in part attributed to their polymeric PEG coatings that prevent nonspecific protein adsorption (opsonisation) and therefore reduce recognition and clearance from the blood by the mononuclear phagocyte system (MPS). PEGylated drug delivery systems typically exhibit prolonged circulation half-lives and improved accumulation at sites of increased vascular permeability when compared to their unmodified counterparts.

The aim of the current study was to examine the potential to translate the materials and concepts that have been successfully employed to enable parenteral stealth applications, to an advantageous role in oral drug delivery. This has been achieved via the systematic evaluation of a series of LBFs with varying degrees of PEG-mediated steric stabilisation. Parenteral stealth formulations aim to evade recognition by the immune system through PEG-mediated prevention of opsonisation. Here, oral 'stealth' formulations are defined as formulations that use a hydrophilic polymeric interfacial layer to similarly prevent nonspecific protein binding, in this case the adsorption of pancreatic lipase-colipase to the surface of a lipid droplet. In this way, the proposed oral stealth LBF aim to evade lipolysis and in doing so prolong drug solubilisation in the GIT and improve drug absorption.

The development of self-assembled stealth LBFs was informed by the structural principles that govern effective stealth attributes in parenteral formulations, namely, surfactant PEG chain molecular weight (Mw) and PEG chain density [26–31]. Here we hypothesised that if oral stealth LBFs behaved in a similar fashion to their parenteral counterparts, digestion inhibition would correlate with the Mw of the surfactant PEG headgroup and the density of the PEG layer. The degree of PEG-mediated reduction in non-specific protein adsorption (as manifest by changes in lipid digestion) was assessed via *in vitro* lipolysis experiments. Changes in drug solubilisation and supersaturation were measured for selected formulations using danazol as a model drug, and these formulations were subsequently administered orally to male Sprague–Dawley rats. Danazol bioavailability was compared after administration of the stealth formulations and structurally analogous formulations that were readily digested.

2. Methods

2.1. Materials and reagents

Danazol (pregna-2,4-dien-20-yno[2,3-d]isoxazol-17-ol) was supplied by Coral drugs PVT (New Delhi, India). Progesterone and 1-aminobenzotriazole (ABT) were from Sigma-Aldrich (St. Louis, MO, USA). Captex® 355 (C_8 and C_{10} MCT) was donated by Abitec Corporation (Janesville, WI, USA) and was used as received. Etocas™ 5, 15, 35, 200 (PEGylated castor oils—CO); Croduret™ 7, 25, 40 (PEGylated hydrogenated castor oils—HCO); Myrj™ S8, 20, 40, 50, 100 (PEGylated stearic acids); Brij™ S2, 10, 20, 100, 200 (PEGylated stearyl alcohols); and Brij™ O2, 3, 5, 10, 20 (PEGylated oleyl alcohols) were kindly donated by Croda International PLC (Yorkshire, England). Jeechem® CAH 16 (PEG 16 hydrogenated castor oil) and Jeechem® CA 25 (PEG 25 castor oil) were donated by Jeen® International Corporation (Fairfield, NJ, USA). Kolliphor® EL (PEG 35 castor oil) and RH40 (PEG 40 hydrogenated castor oil) were donated by BASF Corporation (Washington, NJ, USA). Nikkol® HCO 100 (PEG 100 hydrogenated castor oil) was donated by Nikko Chemicals Co. Ltd. (Chuoku, Tokyo, Japan). Kolliphor® RH60 (PEG 60 hydrogenated castor oil) was purchased from Ingredients Plus (Notting Hill, VIC, Australia). Soybean oil (C_{18} long chain triglycerides—LCT); Triton™ X15, 165, 305, 705 (PEGylated branched octyl phenols); sodium taurodeoxycholate > 95% (NaTDC); porcine pancreatin (8× USP specification activity); and 4-bromophenylboronic acid (4-BPB) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Lipoid E PC S, (Lecithin from egg, approximately 99% pure phosphatidylcholine (PC)) was from Lipoid GmbH (Ludwigshafen, Germany). Sodium heparin (1000 IU/mL) was obtained from DBL (Mulgrave, VIC, Australia), and normal saline (0.9%) was obtained from Baxter Healthcare (Old Toongabbie, QLD, Australia). Sodium hydroxide 1.0 M, which was diluted to obtain 0.6 M and 0.2 M NaOH titration solutions, was purchased from Merck (Darmstadt, Germany), and water was obtained from a Milli-Q (Millipore, Bedford, MA, USA) purification system. All other chemicals and solvents were of analytical purity or high performance liquid chromatography (HPLC) grade. Hypergrade solvents were used for UPLCMS/MS analysis.

2.2. Formulation preparation

2.2.1. Blank formulations

All formulations were prepared as binary mixtures of MCT (Captex® 355) and surfactant (50/50% w/w). The surfactants used are summarised in Table 1. All lipids and surfactants were heated to 37 °C and mixed prior to use to ensure excipient homogeneity. Semisolid excipients (typically surfactants with PEG Mw 800 and above) were heated to 50 °C prior to use. Formulations were vortexed for 30 s after preparation and equilibrated overnight at 37 °C before use.

2.2.2. Drug-loaded formulations

The equilibrium solubility of danazol in each of the drug-loaded LBFs was determined using previously described methodologies [32,33]. Equilibrium solubility was assessed in triplicate and defined as the value attained when at least three consecutive solubility samples varied by ≤5%. This was typically reached after equilibration times of between 48 and 72 h. Danazol containing formulations had drug incorporated at a loading of 80% saturated solubility (based on measured values at equilibrium at 37 °C; Fig. S6). Danazol was accurately weighed into a glass screw cap vial with the required mass of formulation, vortexed and equilibrated for 24 h prior to assay. Danazol content was confirmed by HPLC assay prior to formulation use [16].

2.3. In vitro dispersion and digestion of formulations

2.3.1. Digestion conditions

For formulation screening experiments, *in vitro* dispersion and digestion were conducted as previously reported by the LFCS Consortium [34,35] (Standard conditions Table 2). For experiments conducted on the formulations that were ultimately progressed into rat bioavailability studies, adjustments to volume, enzyme activity and sample mass were made to better reflect a rat model of *in vitro* lipolysis as previously described by Anby et al. [36] (Rat conditions Table 2).

In vitro dispersion was monitored for 15 min and digestion monitored for 30 min. Lipolysis curves were generated from titration of fatty acids with NaOH. As fatty acids liberated during digestion are likely to be partially ionised, titre values were corrected for the presence of unionised fatty acid by back-titration at pH 9 [37,38]. Titre values were compared to the moles of fatty acid that were expected to be released from the formulation to yield a % digestion value. Details of the total extent of digestion calculations can be found in the supplementary material.