The Effect of Administered Dose of Lipid-Based Formulations on the *In Vitro* and *In Vivo* Performance of Cinnarizine as a Model Poorly Water-Soluble Drug

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ABSTRACT: The influence of varying the amount of lipid co-administered with the drug on drug solubilisation and absorption is poorly understood. In the current study, the effect of lipid dose on the *in vitro* drug distribution is compared with the *in vivo* absorption of cinnarizine (CZ) when formulated using long-chain triacylglyceride (LCT) and medium-chain triacylglycerides (MCT). At a fixed drug–lipid ratio, in the closed *in vitro* model, the drug concentrations in the aqueous phase increased and decreased for MCT and LCT, respectively, with increasing lipid dose. However, *in vivo*, the oral bioavailability (*F*%) of CZ was independent of the quantity of lipid administered for both MCT and LCT, but was higher for LCT (32.1 ± 2.3%) than for MCT (16.6 ± 2.3%). Increasing the quantity of lipid relative to the dose of CZ resulted in an increase in the oral *F*% when the lipid mass was increased from 125 to 250 mg, but was no greater at 500 mg lipid dose. The results confirm the limitations of the *in vitro* model but positively indicate that the use of the rat as a pre-clinical model for studying the bioavailability of poorly water-soluble drugs is not compromised by the mass of formulation administered. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:565–578, 2013

Keywords: lipid-based formulation; poorly water-soluble drugs; lipid-based drug delivery system; medium-chain triacylglyceride; long-chain triacylglyceride; lipids; oral absorption; formulation; bioavailability; *in vitro* model

INTRODUCTION

The oral bioavailability of highly lipophilic poorly water-soluble drugs is often limited by their poor aqueous solubility and slow dissolution in the gastrointestinal (GI) tract. Lipid-based formulations have been an increasingly popular means to improve the solubilisation of lipophilic drugs in the GI tract.[1–3] These formulations typically contain lipids such as triacylglycerides of various chain lengths, as well as co-solvents and surfactants to improve drug solubility and formulation dispersibility.

Lipid-based formulations act to improve the oral bioavailability via a number of mechanisms. The digestion of lipids produces diacylglycerides, monoacylglycerides and fatty acids which combine with endogenous bile salts and phospholipids to generate a complex colloidal mixture containing micelles, vesicles and other self-assembled constructs. The colloidal fluid can act as a high-capacity reservoir in the GI tract for the solubilisation of poorly water-soluble drugs, improving the oral bioavailability by maintaining drug in a dissolved state. Exogenous surface-active agents, often incorporated into lipid-based formulations to facilitate dispersion and digestion, may also contribute to the solubilisation of poorly water-soluble drugs. Another mechanism by which lipid-based formulations improve absorption is the prolongation of residence time in the GI tract.[4,5] It has been suggested that lipids, especially long-chain triacylglycerides (LCT), delay gastric emptying and thus increase transit time of the administered drug, thereby increasing the time over which absorption may occur. Lipid-based formulations may also enhance the oral bioavailability of lipophilic drugs through stimulation of the intestinal lymphatic transport pathway,[1,6,7] increased intestinal wall permeability[8–10] and reduced metabolism and efflux activity.[11,12]

Additional Supporting Information may be found in the online version of this article. Supporting Information

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Experimental approaches to understand the influence of lipid-based formulations on drug solubilisation during digestion have largely utilized an *in vitro* digestion model.[13–15] Previous *in vitro* digestion studies have shown that medium-chain triacylglycerides (MCT) can, on digestion, maintain highly lipophilic drugs in solution at a level which is transiently higher than expected from solubility of the crystalline drug in the post-digestion aqueous phase; the effect is not observed on digestion of LCT formulations.[13] However, subsequent studies have shown that this is highly dependent on the quantity of lipid present *in vitro*, and *in vivo* studies with the same or similar formulations have shown that LCT formulations typically enhance the absorption of model lipophilic drugs compared with MCT.[16,17] Furthermore, Sassene et al.[18] have recently demonstrated that cinnarizine (CZ) may in fact precipitate in an amorphous form during *in vitro* digestion of lipid-based formulations. The implication of this finding is that the precipitate may be more likely to redissolve *in vivo* and therefore precipitation may be less likely to be a limitation to the oral bioavailability. This concept has only been shown *in vitro*, and its significance has not been shown *in vivo* to date.

One complexity of *in vitro* models of lipid digestion is that they lack an absorption mechanism to remove digestion products. This can result in accumulation of digestion products in the *in vitro* medium, and the generation of a residual oil phase that retains a significant proportion of drug. This is most evident when exploring simple lipid solution formulations (e.g. Lipid Formulation Classification System formulations)[19] and when examining the digestion behaviour of larger quantities of lipid. This means that the ability of these models to predict *in vivo* behaviour is often inadequate, particularly in light of the fact that the quantity of lipid administered in *in vivo* oral bioavailability studies, in small animals at least, is often high (e.g. >1 g/kg in rat or mouse studies).

When compared with the quantities dosed in larger species, the quantity of lipid used in both *in vitro* and *in vivo* assessment of lipid-based formulations is therefore crucial to digestion, drug solubilisation and absorption but to this point has not been fully investigated.[17,20] As such, the hypothesis to be examined in the current study was that varying the mass of lipid co-administered with a poorly water-soluble drug will influence both the drug distribution between digestion phases during *in vitro* digestion experiments, and drug absorption and the oral bioavailability *in vivo*. Consequently, the primary aim of the present study was to examine the impact of the mass of co-administered lipid on the *in vitro* digestion behaviour and the oral bioavailability of a model lipophilic poorly water-soluble drug (CZ) after administration of simple triacylglyceride solution formulations. CZ ($\log p = 5.77$)[21] was selected as the model poorly water-soluble drug because the oral bioavailability of CZ is limited by solubilisation and dissolution, and hence CZ is a Class 2 compound according to the Biopharmaceutical Classification System.[22]

Both MCT and LCT were studied as formulation lipids, because of the previously reported differences in *in vitro* and *in vivo* behaviour as excipients, to probe the influence of lipid structure. MCT (Captex® 355; Karlshamns USA, Janesville, Wisconsin) consists mainly of $C_{8–10}$ triacylglycerides, and LCT (soybean oil) consists mainly of C_{18} triacylglycerides. In the first part of the study, lipid solution formulations contained CZ at a fixed proportion to the lipid, and the total mass of formulation that was administered to the *in vitro* model or rat model *in vivo* was varied. This allowed examination of the dose dependence on aqueous phase solubilisation, and whether it correlated with *in vivo* oral bioavailability across a range of formulation masses at a fixed drug–lipid mass ratio. To separate the possible contribution of lymphatic transport of CZ to the oral bioavailability of drug after administration of different lipid formulations, the lymphatic transport of CZ after intraduodenal administration was also determined.

It was also hypothesised that the mass of lipid administered was critical to the mechanism of drug solubilisation and dissolution and that an increase in lipid mass at fixed drug dose should lead to an increase in drug absorption. Consequently, studies were also conducted where the drug–lipid mass ratio was varied. In this case, the dose of drug administered in each MCT and LCT formulation was held constant, but the mass of co-administered lipid was varied.

MATERIALS AND METHODS

Materials

Sodium taurodeoxycholate 99% (NaTDC), porcine pancreatin (activity 8× USP specifications), Trizma maleate, soybean oil, CZ, flunarizine, oleic acid, sodium carboxymethylcellulose, benzyl alcohol, Tween® 80 and glacial acetic acid were obtained from Sigma Chemical Company (St. Louis, Missouri); Captex® 355 (Karlshamns USA) was from Karlshamns USA. Dimethyl sulphoxide (DMSO) and 1 M hydrochloric acid were from Ajax Chemicals, New South Wales, Australia. Sodium chloride, calcium chloride dihydrate (BDH Chemicals, Melbourne, Australia); 4-bromophenylboronic acid (4-BPB) (Fluka, Sigma–Aldrich Chemical Company, Milwaukee, Wisconsin) were all used as received. Lecithin [~60% pure phosphatidylcholine (PC) by HPTLC from egg yolk] was a gift from Pharmacia LKB (Uppsala, Sweden). Lipoid EPCS (~99.2% PC) was from Lipoid GMBH (Ludwigshafen, Germany). Sodium hydroxide (1 M, Titrisol; Merck, Darmstadt, Germany) stock