Drug Solubilization Behavior During *in Vitro* Digestion of Suspension Formulations of Poorly Water-Soluble Drugs in Triglyceride Lipids

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Purpose. The purpose of this study was to characterize the solubilization and precipitation characteristics of a range of poorly water-soluble drugs during the *in vitro* digestion of long-chain or medium-chain triglyceride (TG) lipid suspension formulations.

Methods. TG suspensions of model drugs (present at double their equilibrium solubilities in the respective lipid) were digested *in vitro* and the drug solubilization and precipitation pattern in the resulting digests analyzed.

Results. For griseofulvin, diazepam, and danazol, solubilization of the small mass of drug originally presented in the TG lipid was efficient with only a small proportion of the dose precipitating and being recovered in the pellet phase after digestion of the TG lipid. For the more lipophilic and lipid-soluble drugs (cinnarizine, halofantrine), in which higher drug loadings were possible, significant enhancement in drug solubilization in the postdigestion aqueous phase was not apparent compared with simple TG lipid solutions.

Conclusions. Suspensions of drugs, which are poorly soluble in water and TG lipid, may prove beneficial as the relatively high solubilizing capacity of the colloidal phases produced on TG digestion will likely exceed the mass of drug that could have been administered as a simple lipid solution. However, for more lipid-soluble drugs, suspension formulations may offer little benefit as sufficiently high drug loadings can otherwise be achieved with simple solution formulations that still provide for adequate solubilization after TG digestion.

KEY WORDS: dissolution; drug absorption; lipid-based drug delivery; lipid digestion.

INTRODUCTION

The bioavailability of many poorly water-soluble drugs can be increased when they are coadministered with lipids, either in the form of a fatty meal or a lipid-based delivery system (e.g. lipid solution, emulsion, microemulsion) (1–7). Enhanced dissolution and solubilization of the poorly water-soluble drug in the colloidal digestion phases present in the aqueous domain of the intestinal tract is accepted as an important mechanism in the increased bioavailability (8–11). However, the processes that influence the distribution and solubilization of a drug between the coexisting mixed micellar, vesicular, and oil phases present during lipid digestion are not fully understood, and, in part, this is the basis for lipid-based formulation design being largely empirical.

We previously examined the distribution of a series of selected model drugs between the relevant digestion phases when a lipid solution of the drug was subjected to *in vitro* digestion in a recently developed model system (12). From these studies, it was apparent that the triglyceride lipid solubility of hydrophobic drugs (i.e., those with limited aqueous and lipid solubility) was the principle limitation to the effective design of a lipid-based formulation of such drugs. Indeed, equilibrium solubility measurements suggested that the solubilization capacity of the intestinal colloidal phases produced during intestinal processing of typical lipidic formulations was in excess of the mass of drug that could be dissolved in the vehicle. Hence, the factor limiting formulation of hydrophobic drugs as lipid solutions is their limited solubility and, hence, low drug loading per unit dose. These findings prompted the current study, which was undertaken to assess the concept of preparing suspensions of hydrophobic drugs in lipids to increase the drug load per unit dose. In this paper, the solubilization behavior of the five previously selected poorly water-soluble drugs (griseofulvin, GRIS; diazepam, DIAZ; danazol, DAN; cinnarizine, CIN; and halofantrine, HF) was examined during the *in vitro* digestion of medium-chain and long-chain triglyceride lipid suspensions. Furthermore, as ionizable poorly water-soluble drugs are often prepared as a salt in an attempt to enhance their water solubility (and making them less lipophilic and less lipid-soluble), a lipid suspension of the poorly lipid-soluble HCl salt of HF was also studied as recent data demonstrated facile conversion to the lipophilic and highly lipid-soluble free base of HF at physiological pH (13).

MATERIALS AND METHODS

Materials

Sodium chloride, 1 M hydrochloric acid (Ajax Chemicals, Sydney, Australia); calcium chloride dihydrate (BDH Chemicals, Melbourne, Australia); 4-bromophenylboronic acid (4-BPB) (Aldrich Chem. Co., Milwaukee, WI, USA); sodium taurodeoxycholate 99% (NaTDC), porcine pancreatin (activity 8 × USP specifications), Trizma maleate, soybean oil (SBO) (Sigma Chemical Co., St. Louis, MO, USA); Maisine 35-1 (Gattefossé, Saint-Priest, France); and Captex 355 and Capmul MCM (Abitec Corporation, Janesville, WI, USA) were all used as received. Details of the constituent components of the various lipids have previously been described (12). Lecithin [approximately 60% pure phosphatidylcholine (PC) by HPTLC (14)] was obtained from Pharmacia LKB (Uppsala, Sweden) and was used as received. Stock solution of 1 M sodium hydroxide (Titrisol, Merck, Darmstadt, Germany) was diluted with water to obtain 0.2 and 0.6 M NaOH titration solutions. Solvents were HPLC grade (Mallinckrodt,

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ABBREVIATIONS: 4-BPB, 4-bromophenylboronic acid; BS, bile salt; CIN, cinnarizine; DAN, danazol; DG, diglyceride; DIAZ, diazepam; FA, fatty acid; GRIS, griseofulvin; HF, halofantrine base; HF·HCl, halofantrine hydrochloride; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; MG, monoglyceride; NaTDC, sodium taurodeoxycholate; PC, phosphatidylcholine; PL, phospholipid; SBO, soybean oil; TBU, tributyrin units; TG, triglyceride.

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Paris, KY, USA) and water was obtained from a Milli-Q (Millipore, Billerica, MA, USA) water purification system. Griseofulvin, cinnarizine (Sigma); diazepam (Alphapharm, Glebe, Australia); danazol (DAN, Sterling Pharmaceuticals, Sydney, Australia); halofantrine base and halofantrine hydrochloride (GlaxoSmithKline, Mysore, India) were used as received.

Solubility Studies

The equilibrium solubility of HF·HCl was determined in long-chain triglyceride (LCT; soybean oil), medium-chain triglyceride (MCT; Captex 355), in glyceride mixtures representing partially digested triglycerides (Maisine 35-1 and Capmul MCM), and in bile salt (BS)/phospholipid (PL) solutions representative of the BS/PL concentrations encountered in the fasted (5 mM NaTDC/1.25 mM PC) (15,16) and postprandial intestine (20 mM NaTDC/5 mM PC) (15,17). BS/PL solutions were prepared in the buffer used to conduct digestion experiments (tris-maleate 50 mM, 150 mM NaCl, 5 mM $CaCl_2 \cdot 2H_2O$, pH 7.5) as previously described (14). Equilibrium solubility of each model drug was also determined in a "blank" aqueous phase obtained by digestion of 250 mg of the two triglycerides (MCT and LCT) under high and low BS/PL conditions.

In vitro Digestion of Lipid Suspensions

In vitro digestion experiments were conducted as previously described (12,14). Briefly, known quantities of lipid (containing suspended drug at 200% of saturated solubility in the respective lipid) were dispersed by stirring in 9 ml of digestion buffer containing either low (5 mM NaTDC/1.25 mM PC) or high (20 mM NaTDC/5 mM PC) concentrations of BS and PL. Experiments were performed at 37°C in a stirred, thermostatted glass vessel and digestion was initiated by the addition of 1 ml of a pancreatin extract [containing 10,000 tributyrin units (TBU) of pancreatic lipase activity]. The pancreatin extract was prepared as previously described (14). Digests were performed using a pH-stat titration unit (Radiometer, Copenhagen, Denmark), which maintained the pH at 7.5 for the period of the experiments. The FA produced by digestion of the TG was titrated with 0.2 M NaOH for LCT (soybean oil) digests and 0.6 M NaOH for MCT (Captex 355) digestion studies. Digestion was conducted over either 30 or 60 min, after which 2 × 4 ml aliquots of the postdigestion mixture were ultracentrifuged (334,000*g*, 30 min, 37°C, Optima XL-100K, SW-60 rotor, Beckman, Palo Alto, CA, USA) to separate the digest into an oil phase, an aqueous phase, and a pellet phase. Blank aqueous phases were produced for equilibrium solubility measurements by repeating the digests with drug-free lipids. Samples obtained from digestion experiments (oil, aqueous, and pellet phases) were assayed by HPLC as previously described (12).

To examine the impact of increasing drug loading within the triglyceride suspension on the subsequent solubilization of drug within the postdigestion milieu, digests were conducted in 5 mM NaTDC/1.25 mM PC with suspensions of GRIS in MCT and LCT at concentrations between 1 and 30 mg per gram of lipid which represent drug concentrations between 2- and 10-fold the equilibrium solubility of GRIS in the respective lipids.

RESULTS

Drug Solubility in the Formulation Lipids

The (mean ± SD, n = 3) solubility of the five model drugs in LCT was previously determined to be 0.48 ± 0.01 mg/g (GRIS), 18.3 ± 0.2 mg/g (DIAZ), 3.90 ± 0.05 mg/g (DAN), 27.0 ± 0.6 mg/g (CIN), and 47.3 ± 6.5 mg/g HF, with the values in MCT being 0.95 ± 0.03 mg/g (GRIS), 30.3 ± 0.5 mg/g (DIAZ), 8.7 ± 0.1 mg/g (DAN), 40.7 ± 0.3 mg/g (CIN), and 89.0 ± 2.7 mg/g (HF) (12). These values were used to determine the drug loading level to prepare the lipid suspensions, which were designed to contain drug at 200% of its equilibrium solubility in the respective lipid at 37°C.

The solubilities of HF·HCl determined at 37°C in both MCT and LCT and their respective MG/DG variants (Capmul MCM and Maisine, respectively) are given in Table I, with previously determined data for HF base included for comparative purposes (12). As expected, the solubility of the hydrochloride salt of HF in the TG lipids was significantly less than the free base form. The solubility of HF·HCl in Maisine and Capmul was significantly higher compared with the respective "parent" triglyceride, and surprisingly, at least in the case of Capmul, the value was higher than that observed for HF free base.

Table I. Mean (± SD, n = 3) Equilibrium Solubilities Determined at 37°C for HF Base and HF·HCl in a Long-Chain Triglyceride, a Long Chain Glyceride Blend, a Medium-Chain Triglyceride, a Medium-Chain Glyceride Blend, and in Digestion Buffer and Low (5 mM NaTDC/1.25 mM PC) and High (20 mM NaTDC/5 mM PC) BS and PL Micellar Solutions

	Equilibrium solubilities					
Solvent composition	HF base* (mg/g)	HF·HCl (mg/g)	HF base* (mmol/mol)	HF·HCl (mmol/mol)	HF base* (μg/ml)	HF-HCl (μg/ml)
Soybean oil (LCT)	47.3 ± 6.5	0.07 ± 0.01	82.3 ± 11.4	0.12 ± 0.02		
Maisine (long-chain MG/DG)	49.1 ± 3.7	11.4 ± 0.3	53.0 ± 3.9	12.3 ± 0.3		
Captex 355 (MCT)	89.0 ± 2.7	0.19 ± 0.01	89.7 ± 2.8	0.19 ± 0.01		
Capmul MCM (medium-chain MG/DG)	26.1 ± 2.4	40.4 ± 2.5	14.5 ± 1.3	22.4 ± 1.4		
Digestion buffer (pH 7.5)					<0.1	0.2
5 mM BS/1.25 mM PL micellar solution					12.6 ± 0.1	180.8 ± 1.4
20 mM BS/5 mM PL micellar solution					60.7 ± 0.02	476.0 ± 53.0

HF·HCl, halofantrine hydrochloride; NaTDC, sodium taurodeoxycholate; PC, phosphatidylcholine; BS, bile salt; PL, phospholipid; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; MG, monoglyceride; DG, diglyceride.
* Data for HF base are reproduced from Ref. 12.

Rate and Extent of Digestion of Lipid Suspensions

The rate and extent of digestion of the lipid suspension formulations over the 30-min period of digestion were essentially identical (data not shown) to that previously described for TG solution formulations containing the same drugs (12) and pure TG lipids (18) where the extent of LCT digestion (expressed as the percentage loss of TG after 30 min) was 42.1% under low BS/PL conditions and 60.6% under high BS/PL conditions, and the digestion of the MCT was essentially complete under both conditions. Therefore, any differences in drug distribution between the currently reported lipid suspensions and the previously reported lipid solutions can be attributed to different drug solubilization and trafficking effects and not to differences in digestion profiles or different lipid digestion products/phases present in the postdigestion aqueous phase.

Consistent with the previous lipid solution studies (12,18), the LCT suspension formulations were partially digested over the 30-min digestion period with the three phases present after ultracentrifugation being an oil phase containing principally undigested triglyceride, an aqueous phase containing mixed micelles and larger colloidal species which were presumably vesicles and/or liquid crystals (17,19–21), and a solid pellet. In contrast to the LCT suspensions, digestion of the MCT suspension formulations was complete and there was no residual oil phase present after the 30 min digestion.

Drug Solubilization and Distribution Profiles

The pattern of the solubilization and distribution of the poorly water-soluble drugs in the postdigestion phases resulting from the lipid suspensions (formulated in either 250 mg LCT or MCT and present at 200% of their respective equilibrium lipid solubilities) are presented in Fig. 1. The drug distribution data are presented as a function of the individually determined drug distribution coefficient between a 5 mM NaTDC/1.25 mM PC micellar solution and LCT or MCT, respectively, as this value better represents the partitioning phenomena occurring during digestion (as opposed to an octanol/water distribution coefficient) (12).

For the LCT suspension formulations, the distribution pattern of the different drugs between the various postdigestion phases was similar to those previously reported for lipid solution formulations of the same drugs (where drug was present at 50% of its solubility in the lipid). Not surprisingly, the principal difference between the current suspension data and the previous solution formulation data was that a higher proportion of the initial drug load was recovered in the pellet in the case of the suspensions. For the LCT suspension formulations, 2–20% of the drug was recovered in the pellet after ultracentrifugation compared with 2–4% in the case of the lipid solutions (12). For the MCT suspensions of GRIS and DIAZ, the distribution data were similar to the previous distributions obtained using lipid solutions. However, for the highly lipophilic drugs CIN and HF, a much greater proportion of the initial drug load (88–93%) was recovered in the postdigestion pellet from the lipid suspensions compared with the lipid solutions.

Although in many cases a lower proportion of the total drug present in the lipid suspension distributed into the aqueous phase of the lipid digests (Fig. 1), when compared with

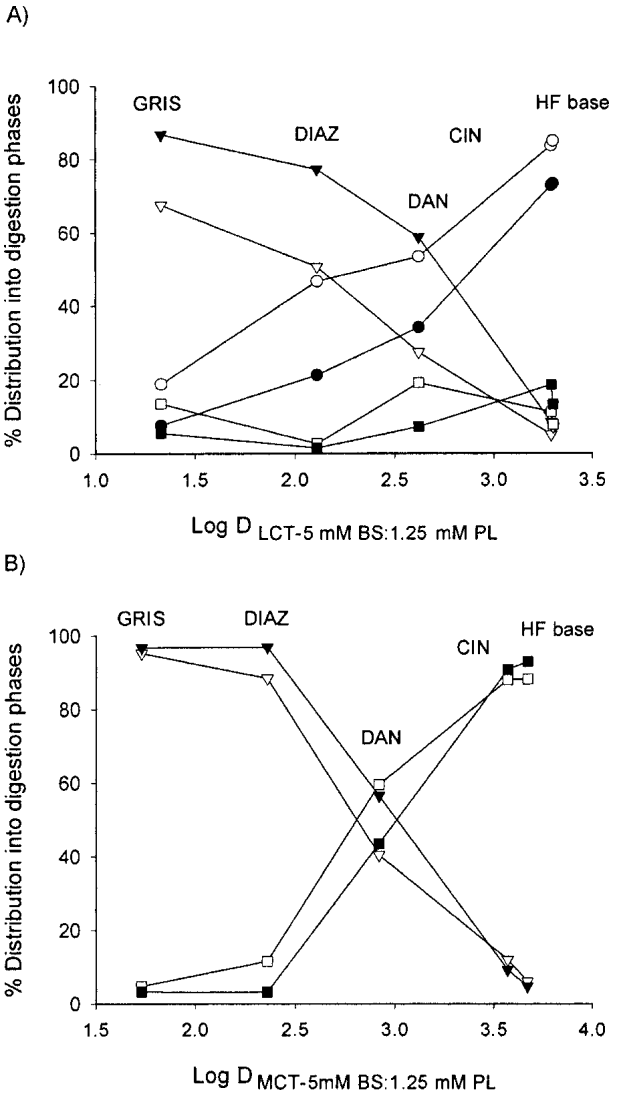

Fig. 1. Distribution of griseofulvin (GRIS), diazepam (DIAZ), danazol (DAN), cinnarizine (CIN), and halofantrine (HF) into a nondispersed oil phase (●, ○), dispersed aqueous phase (▼, ▽), and pellet phase (□, ■) after 30 min digestion of 250 mg of long-chain triglyceride (LCT, panel A) or medium-chain triglyceride (MCT, panel B) under high (20 mM NaTDC/5mM PC, filled symbols) and low (5 mM NaTDC/1.25 mM PC, open symbols) bile salt and phospholipid conditions. Drugs were suspended in LCT and MCT at 200% of their individual saturated solubilities.

the previous lipid solution data, the actual concentrations of drug attained in the aqueous phase were generally higher when compared with the lipid solution data which presumably reflected the higher initial drug loadings (Table II). The relative increase in aqueous phase concentration afforded by increasing the drug load in the lipid suspensions is shown in Table II. Interestingly, for GRIS and DIAZ, the increase in observed aqueous phase concentration was directly proportional to the increased initial drug loading. For DAN, proportional increases in the aqueous phase concentration were observed for the suspension in LCT, but not with MCT. For the highly lipophilic CIN and HF, higher aqueous phase concentrations were attained during digestion of the LCT suspensions; however, this increase was not proportional to the increased drug load in the suspension. Furthermore, digestion

Table II. Measured Aqueous Phase Concentrations of Drug [Aq phase] Obtained After Digestion of Selected Drug Formulations Prepared as Either Suspensions or Solutions in LCT or MCT Lipids Under Low and High BS/PL Conditions*

		Griseofulvin		Diazepam		Danazol		Cinnarizine		Halofantrine base	
Digestion conditions		[Aq phase] (μg/ml)	Saturation (%)†	[Aq phase] (μg/ml)	Saturation (%)†	[Aq phase] (μg/ml)	Saturation (%)†	[Aq phase] (μg/ml)	Saturation (%)†	[Aq phase] (μg/ml)	Saturation (%)†
LCT digest	Suspension	14.4	26.9	404.8	78.9	47.0	76.5	65.8	30.8	182.5	51.5
(5 mM BS/	Solution	4.0	7.5	111.4	21.7	13.8	22.5	26.1	12.2	72.0	20.3
1.25 mM PL)	[Aq] ratio‡	3.6		3.6		3.4		2.5		2.5	
	Dose ratio§	3.7		4.0		4.0		4.6		3.4	
MCT digest	Suspension	36.0	38.4	1136	147	126.7	123.2	107.1	56.8	566.5	205.9
(5 mM BS/	Solution	9.2	9.8	271.9	35.0	83.1	80.8	279.5	148.2	756.1	274.8
1.25 mM PL)	[Aq] ratio‡	3.9		4.2		1.5		0.4		0.8	
	Dose ratio§	3.7		4.1		4.1		4.2		4.1	
LCT digest	Suspension	17.4	15.0	580.8	56.0	93.5	65.6	105.2	56.0	306.9	28.6
(20 mM BS/	Solution	5.6	4.8	143.2	13.8	23.8	16.7	35.9	19.1	109.9	10.3
5 mM PL)	[Aq] ratio‡	3.1		4.1		3.9		2.9		2.8	
	Dose ratio§	3.4		4.1		4.1		4.8		3.5	
MCT digest	Suspension	37.0	26.1	1199	119	179.1	133.6	88.5	38.4	404.8	127.3
(20 mM BS/	Solution	9.2	6.5	280.7	27.8	81.3	60.6	227.7	98.8	401.8	126.4
5 mM PL)	[Aq] ratio‡	4.0		4.3		2.2		0.4		1.0	
	Dose ratio§	3.7		4.2		4.1		4.2		4.2	

LCT, long-chain triglyceride; MCT, medium-chain triglyceride; BS, bile salt; PL, phospholipid.
* The data for the lipid solutions are taken from Ref. 12 and are provided for comparative purposes.
† Percentage saturation is the [Aq phase] at the end of the digestion expressed as a percentage of the equilibrium solubility in the Aq phase obtained from a "blank" lipid digest.
‡ Ratio of the [Aq phase] attained at the end of the digestion for lipid drug suspensions compared with the lipid drug solutions.
§ Actual ratio of drug loading in the suspension formulation compared with the solution formulation (the nominal value was 4.0).

of the MCT lipid suspensions resulted in lower aqueous phase drug concentrations than previously observed with the lipid solutions, which contained a 4-fold lower concentration of drug (12).

GRIS Lipid Suspensions: Effect of Different Drug Loadings

To study further the solubilization profile of drugs that are poorly soluble in triglyceride lipids, suspensions of GRIS were prepared at increasing drug loadings and then subject to digestion under low BS/PL conditions (Table III and Fig. 2).

Increasing the concentration of GRIS in the TG lipid from solution conditions (GRIS at 50% its equilibrium solubility) to then produce a suspension (GRIS at 2-fold its equilibrium solubility) led to a linear increase in the concentration of GRIS attained in the aqueous digestion phase from either the MCT or LCT lipid. Further increases in drug loading in the suspensions to 7.5-fold the equilibrium solubility in MCT

Table III. Concentration of Griseofulvin in the Aqueous Phase [Aq phase] and the Percentage Distribution of Griseofulvin into the Digestion Phases after 30 Min Digestion of Either Lipid Solution or Lipid Suspensions*

Lipid	Drug load (mg/digest)	[Drug] in lipid (mg/g)	% drug solubility in lipid†	Relative drug load	[Aq phase] (μg/ml)	Increase in [Aq phase]	% distribution into Aq phase	% distribution into oil phase	% distribution into pellet phase
Soybean oil (LCT)	0.068‡	0.27	56	1	4.0	1	75.0	21.5	<3.0
	0.248	0.994	207	3.7	14.4	3.6	67.5	18.9	13.5
	0.597	2.36	492	8.8	37.1	9.3	64.7	20.1	18.2
	1.14	4.54	946	16.9	36.9	9.2	36.5	10.0	53.6
	1.774	7.08	1480	26.3	39.4	9.9	24.2	3.3	72.5
Equilibrium solubility					53.6	± 1.8			
Captex 355 (MCT)	0.130‡	0.54	57	1	9.2	1	97.2	—	2.8
	0.477	1.98	208	3.7	36.0	3.9	95.2	—	4.8
	1.777	7.11	748	13.7	101.3	11.0	61.4	—	38.6
	3.541	13.7	1442	27.3	113.9	12.4	43.0	—	57.0
	8.423	33.4	3516	64.9	96.1	10.4	23.0	—	77.0
Equilibrium solubility					93.8	± 2.1			

PC, phosphatidylcholine; BS, bile salt; PL, phospholipid; LCT, long-chain triglyceride; MCT, medium-chain triglyceride.
* The digestions were performed under low (5 mM NaTDC/1.25 mM PC) BS and PL conditions (mean ± SD, n = 3).
† Drug present either as a solution in each lipid (i.e., where drug present at <100% of drug solubility in the lipid) or as a suspension of drug in lipid (where drug present at >100% of drug solubility).
‡ Data at this concentration of griseofulvin are reproduced from Ref. 12.

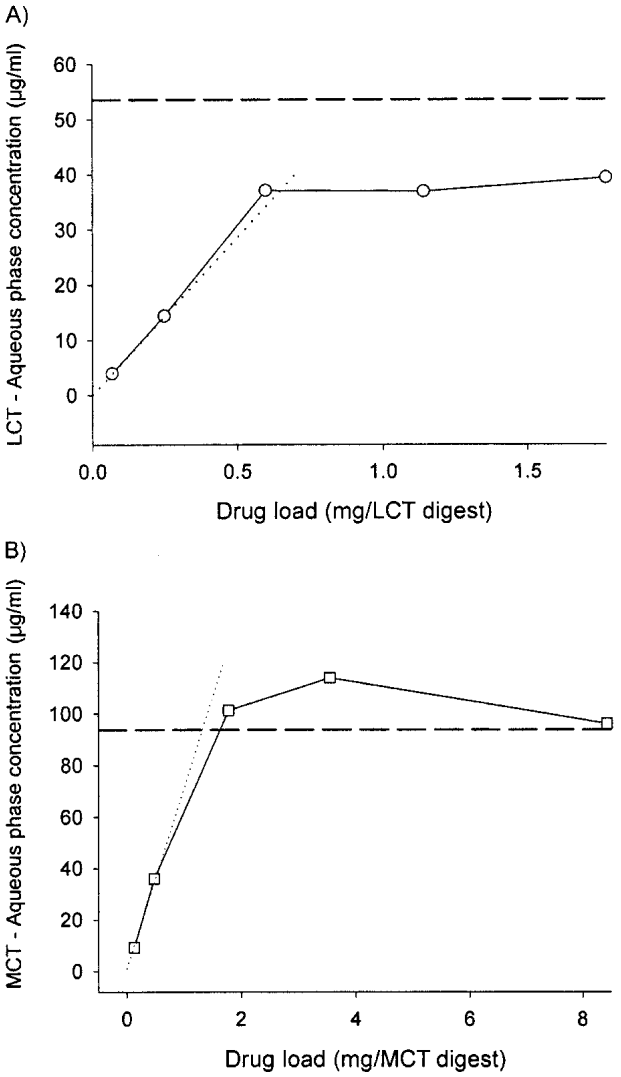

Fig. 2. The attained aqueous phase concentrations, as a function of increasing drug load of griseofulvin (GRIS), after 30 min digestion of 250 mg of long-chain triglyceride (○, LCT, panel A) or medium-chain triglyceride (□, MCT, panel B) under low (5 mM NaTDC/1.25 mM PC) bile salt and phospholipid conditions. The dashed line (- - -) represents the equilibrium solubility of GRIS determined in the respective blank aqueous phase (from Ref. 12). and the dotted line (···) represents the extrapolated linear increase in aqueous phase concentration as a function of increasing drug load.

lipids and 5-fold in LCT lipids led to approximately proportional increases in aqueous phase concentrations in the digest milieu; however, further increases in drug load did not substantially increase the aqueous phase concentration attained. In the case of the MCT suspensions, increases in drug loading appeared to result in linear increases in aqueous phase concentrations until the aqueous digestion phase became saturated (based on the equilibrium solubility of GRIS in blank aqueous phase obtained by digestion of an identical quantity of drug-free lipid). In the LCT suspensions, the presence of the undigested LCT under the experimental conditions limited attainment of aqueous phase concentrations approaching the equilibrium solubility, although concentrations of approximately 75% of saturation were achieved.

Distribution Profiles for HF Base vs. HF·HCl Suspensions

The distribution profiles and the aqueous phase concentrations of HF (expressed as equivalents of HF base) obtained from digestion of HF·HCl suspensions (HF·HCl suspension present at a drug load corresponding to 50% of the equilibrium solubility of HF base in each lipid) are described in Table IV. Despite the HF·HCl suspensions containing masses of drug several orders of magnitude higher than the equilibrium solubility of HF·HCl in the respective lipids, the distribution profiles and aqueous phase concentrations of HF obtained at the end of a 30-min digestion period were similar to those obtained after digestion of lipid solutions containing HF base. Similar data were also obtained when digests were performed using MCT and LCT suspensions of HF·HCl and HF base where drug was present at twice the equilibrium solubility of the base in each of the lipids (i.e., at 4-fold higher drug loads) (data not shown).

DISCUSSION

Lipid-based formulations have been shown to enhance the oral bioavailability of various poorly water-soluble, lipophilic drugs including cyclosporine (22), saquinavir (23), ritonavir (23), halofantrine (24), and danazol (4). The utility of such formulations is typically ascribed to enhanced drug dissolution and increased solubilization due to the formation of colloidal intestinal phases derived from exogenous formulation components, lipid digestion products, and biliary-derived bile salts and phospholipids.

In a previous study, we examined the *in vitro* postdigestion solubilization of a range of poorly water-soluble drugs after formulation in simple triglyceride lipids where it was demonstrated that substantial improvements in solubility could be attained in the presence of a digesting lipid (12). For hydrophobic drugs (i.e., those with limited aqueous and lipid solubility), it was not the solubilizing capacity of the formed colloidal systems that precluded attainment of high aqueous phase drug concentration, but rather the limited mass of drug that could be dissolved within the triglyceride lipid solutions. It was this observation that stimulated the current investigation into suspensions of poorly water-soluble drugs as a means to circumvent the inherent dose limitations associated with lipid solution formulations.

For the lower log *P* drugs such as GRIS and DIAZ, digestion of either MCT or LCT lipid suspensions resulted in dose-dependent increases in the drug concentration attained in the aqueous phase obtained after ultracentrifugation (Table II). For these drugs, the overall solubilization capacity of the digestion phases was greater than the amount of drug that could have otherwise been dissolved within either a MCT or LCT lipid solution, and hence, suspensions may offer a viable formulation strategy for supporting and enhancing overall absorption. Further studies with GRIS demonstrated, at least in the closed *in vitro* system, that rapid equilibration of drug concentration across the lipid and aqueous phases occurred and that suspensions of increasing drug load could usefully be used to the point at which the equilibrium solubility of the drug in the aqueous phase formed was reached (Table III; Fig. 2). For example, GRIS lipid suspensions containing greater than 7-fold the maximal drug load that could be achieved in a lipid solution led to proportional increases in

Table IV. Concentration of Halofantrine in the Aqueous Phase [Aq phase] and the Percentage Distribution of Halofantrine into the Various Digestion Phases after 30 Min Digestion of Either Lipid Solutions of HF Base or Lipid Suspensions of HF·HCl Prepared in Either Soybean Oil (LCT) or Captex 355 (MCT)*

Digestion conditions	Halofantrine†	[Drug] in lipid (mg/g)	[Aq phase] (μg/ml)	% distribution into Aq phase	% distribution into oil phase	% distribution into pellet phase
Soybean oil digest (LCT)	Base	27.0 ± 0.9	72.0 ± 0.2	11.5 ± 0.9	86.6 ± 0.9	2.0 ± 0.1
(5 mM BS/1.25 mM PL)	HCl	27.4	63.9	10.2	86.1	3.8
Captex 355 digest (MCT)	Base	44.5 ± 0.5	756.1 ± 45.6	82.7 ± 2.7	—	17.3 ± 2.7
(5 mM BS/1.25 mM PL)	HCl	46.9	769.0	86.0	—	14.0
Soybean oil digest (LCT)	Base	27.2 ± 0.5	109.9 ± 5.7	18.5 ± 2.1	78.2 ± 1.8	3.3 ± 0.4
(20 mM BS/5 mM PL)	HCl	26.9	124.7	22.0	71.1	6.9
Captex 355 digest (MCT)	Base	42.4 ± 3.8	401.8 ± 45.3	47.4 ± 3.3	—	52.6 ± 3.3
(20 mM BS/5 mM PL)	HCl	46.9	386.5	41.2	—	58.8

HF·HCl, halofantrine hydrochloride; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; NaTDC, sodium taurodeoxycholate; PC, phosphatidylcholine; BS, bile salt; PL, phospholipid.

* The digestions were performed under low (5 mM NaTDC/1.25 mM PC) and high (20 mM NaTDC/5 mM PC) BS and PL conditions. Data are presented as mean ± SD (n = 3) where indicated.

† HF base formulations were prepared at 50% of the saturated solubility in the respective lipid (i.e., as drug solutions in the lipid). The HF·HCl suspensions were prepared at an equivalent drug loading of the HCl salt in each lipid and were suspensions of drug in lipid. HF base data are reproduced from Ref. 12.

the aqueous phase concentrations attained at the end of a 30-min digestion period.

For the higher log *P* drugs CIN and HF, the potential benefit of formulation as a lipid suspension was less evident. Indeed for the MCT systems, the lipid suspension formulations led to lower concentrations of drug present in the aqueous phase after 30 min digestion compared with previous data obtained using drug lipid solutions at 4-fold lower doses (Table II). It is likely that the relatively poor performance of the lipid suspension formulations stems from the fact that whereas digestion of lipid solutions was previously shown to support supersaturation of the aqueous phase, the presence of solid drug in the suspension formulations most likely limited achievement of a supersaturated solution thereby reducing the attainable drug concentration.

Increasing the drug load in the lipid formulation by forming a suspension led to small nonlinear increases in the LCT-derived aqueous phase concentration for CIN and HF. Interestingly, low amounts of HF and CIN were recovered in the pellet phase after digestion of the LCT lipid and the drugs appeared to concentrate/accumulate within the remaining incompletely digested oil phase. For example, after 30 min digestion of a HF suspension (250 mg LCT, HF present at 200% of its saturated solubility in the lipid) under low BS and PL conditions (5 mM NaTDC/1.25 mM PC), 87% of the initial HF was present in the remaining oil phase. Even assuming that the mass of undigested LCT remained unchanged from the initial starting conditions (which is an underestimation as substantial digestion occurs), it is apparent that supersaturation of the oil phase occurred. When further experiments were undertaken using lower lipid loads (50 mg lipid with HF present at 2-fold its solubility in lipid) under circumstances where complete digestion occurred (60 min digestion under 20 mM NaTDC/5 mM PC conditions), it was evident that as digestion progressed, the HF became concentrated within the oil phase, but that on complete digestion approximately 70% of the dose precipitated (as opposed to partitioned into the aqueous phase). This occurred in spite of the HF concentration in the aqueous phase at the end of digestion (152.4 μg/ml) being substantially lower than the equilibrium solubility in the simulated aqueous phase (221.7 ± 7.2 μg/ml). This most likely reflects a rapid "dose dumping" event when digestion of the lipid approaches completion (i.e., at the point at which the coexisting lipid reservoir is depleted entirely)—with the kinetics of the process precluding efficient redistribution into the aqueous phase even though the aqueous phase concentration was below saturation.

Mixed results were obtained for DAN where linear increases in aqueous phase concentration were attained for the LCT systems (similar to GRIS and DIAZ), while for the MCT formulations only a relatively small increase (1.5- to 2.2-fold increase) in the aqueous phase concentrations was evident (Table II).

For many poorly water-soluble acidic or basic drugs, early development efforts typically pursue progression based on aqueous solubility (and not lipid solubility) via the preparation of salts. In terms of a lipid-based formulation, however, it is typically the lipid-soluble free base or acid, rather than the salt, that is most amenable to formulation development due to advantageous solubilities in lipids and related excipients. To examine the differential behavior of neutral and salt forms of the same drug, digests were performed with HF free base and HF·HCl. As is evident in Table I, the lipid solubility of HF·HCl was substantially lower than the free base (with the exception of HF·HCl solubility in Capmul which was surprisingly high). Digests were performed with MCT or LCT containing HF·HCl as a suspension, at the same concentration as that previously used for HF base solutions (where HF base was present at 50% of its solubility in the respective lipid). Surprisingly, almost identical HF distribution patterns were observed within the lipid phases that formed postdigestion, and the amount of HF recovered in the oil phase and the concentration in the aqueous phase measured at the end of the digest were also effectively the same (Table IV). These data suggest rapid interconversion of HF·HCl to HF base followed by distribution of the free base into the oil phase during digestion. This conclusion is supported by the previously reported behavior of HF·HCl during equilibration between SBO and a mixed micellar solution (15 mM taurocholate/3.75 mM lecithin) where FTIR analysis of the HF species

present in the oil at the end of the digestion period confirmed the presence of HF base (13). It is apparent that rapid conversion of HF·HCl to the more lipid-soluble base can readily occur (even in the absence of digestion), although solubilization of HF·HCl is expected to be enhanced/accelerated by the digestion process as more polar glycerides are formed. Therefore, HF·HCl is effectively "trafficked" out of the digesting TG lipid into both the MG and DG digestion products and the BS/PL aqueous phase (where the solubility of HF·HCl in BS/PL micelles is higher than that of HF base) where rapid equilibration to the free base then occurs. The HF base, so formed, subsequently partitions across the various colloidal phases in a similar fashion to that which would be expected had a solution or suspension of HF base been initially administered.

In summary, for drugs with poor aqueous and lipid solubility, formulation as suspensions in lipids may prove beneficial as the solubilization capacity of the colloidal phases produced on digestion of the lipid vehicle is likely to be greater than the dose of drug that could otherwise be administered as a lipid solution. Clearly, the use of suspension formulations presents its own challenges including particle size changes, uniformity of dispersion, and viscosity issues; however, these may be easier to overcome than the intrinsic (and common) problems of low potential dose in lipid solution preparations. For more lipophilic drugs, suspensions may assist, but the benefit is unlikely to be as significant, as the dose of drug that can be administered as a lipid solution is substantially higher. Finally, for weak acids or bases with pK_as approaching luminal pH, the solubilization pattern of the salt is likely to mirror that of the free acid/base because partitioning behavior will reflect the species with the greatest affinity for each phase. This approach may maximize luminal solubility by promoting solubilization within the dispersed lipidic microdomains (via the free acid/base) and enhancing aqueous solubility (via availability of the ionized species).

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