Pharmaceutical Research, Vol. 21, No. 8, August 2004 (© 2004) *Research Paper*

Susceptibility to Lipase-Mediated Digestion Reduces the Oral Bioavailability of Danazol After Administration as a Medium-Chain Lipid-Based Microemulsion Formulation

Christopher J. H. Porter,[1,4] Ann Marie Kaukonen,[1,3] Ben J. Boyd,[1] Glenn A. Edwards,[2] and William N. Charman[1]

Received March 10, 2004; accepted April 28, 2004

Purpose. To investigate the impact of lipidic formulation type on *in vitro* dispersion and digestion properties and the relationship to oral bioavailability, using danazol as a model lipophilic poorly water-soluble drug.

Methods. Three lipid-based danazol formulations [a long-chain triglyceride solution (LCT-solution) and self-microemulsifying drug delivery systems (SMEDDS) based on long-chain (C_{18}) lipids (LC-SMEDDS) and medium-chain (C_8–C_{10}) lipids (MC-SMEDDS)] were administered to fasted beagle dogs and compared with a micronized danazol formulation administered postprandially and in the fasted state. *In vitro* dispersion and particle size data for the two SMEDDS were compared, and the distribution/solubilization patterns of danazol across the various phases produced during *in vitro* digestion quantified.

Results. The LCT-solution and LC-SMEDDS formulations significantly enhanced the oral bioavailability of danazol when compared to fasted administration of the powder formulation. In contrast, and despite displaying excellent dispersion properties, the MC-SMEDDS resulted in little enhancement in danazol bioavailability. In support of the *in vivo* findings, i*n vitro* digestion of the medium-chain formulation resulted in significant drug precipitation when compared with the long-chain lipid formulations.

Conclusions. Digestion of microemulsion preconcentrate formulations based on medium-chain lipids may limit *in vivo* utility when compared with similar formulations based on long chain lipids.

KEY WORDS: absorption bioavailability; dispersion; lipid-based drug delivery; lipid digestion; poorly water-soluble drugs.

INTRODUCTION

Lipid-based formulations may significantly enhance the oral bioavailability of poorly water-soluble drug molecules (1,2). Although an overall rationale for such enhancement has not been forthcoming, it is generally thought to stem from an increase in *in vivo* dissolution. More specifically, the increase in dissolution rate results from the administration of drug in a predissolved state (typically in a lipid microenvironment, such as a lipid solution, emulsion, or microemulsion), thereby reducing the energy associated with a solid-liquid phase transition, and from increased drug solubilization in the colloidal structures that are produced by the interaction of the formulation and its digestion products with endogenous biliary amphiphiles such as bile salts and phospholipids (3,4).

Most recently, there has been an increasing focus on the utility of lipidic microemulsion formulations, or more correctly, microemulsion precursor solutions, that consist of an isotropic mixture of drug, lipid, surfactant, and typically a co-surfactant or co-solvent. When exposed to the fluids of the gastrointestinal (GI) tract, these precursor solutions spontaneously emulsify to form highly dispersed microemulsions (5,6). These dispersions commonly exhibit particle sizes below 100 nm and have been shown to enhance the oral bioavailability of lipophilic drugs such as cyclosporine (7), halofantrine (8), ontazolast (9), and progesterone (2). The ease of dispersion and the very small particle size of the resultant colloidal microemulsion have historically been viewed as the principal reasons for their utility in the delivery of lipophilic drugs (10). Consequently, most of the commercially available lipidic formulations are complex mixtures of lipids, surfactants, and co-solvents/co-surfactants constructed to both improve drug solubility in the formulation (and therefore increase drug payload) and also to maximize dispersion of the dose form on exposure of the capsule fill to the GI contents. The design, development, and testing of these formulations are often based entirely on these formulation end-points—namely drug solubility in the dose form and rapid and complete dispersion in a simple *in vitro* dispersion test (commonly coupled with a particle size measurement to define the resulting dispersion).

In a series of previous publications, we have examined the factors that affect the solubilization of poorly water-soluble drug molecules after co-administration with lipidic vehicles using an *in vitro* lipid digestion model (11–13). These studies demonstrated that *in vitro* digestion models, which include an ultracentrifugation step to separate the digest into an aqueous dispersed phase (from where drug absorption most likely occurs), a pellet phase, and an undigested/undispersed lipid phase, are a useful tool with which to predict the likely pattern of drug solubilization *in vivo*. An important aspect of these previous studies was the understanding that administration of lipophilic drugs in combination with triglycerides composed of medium chain (C_8–C_{10}) length lipids, may provide less suitable solubilization conditions because the digestion of these triglycerides leads to the production of medium-chain fatty acids and monoglycerides, the capacity of which to swell biliary micellar structures is considerably reduced when compared with longer chain (C_{14}–C_{18}) lipids (3,13). However, analogous studies into the effects of digestion on more complex self-microemulsifying systems and the potential ramification of the digestion of these systems on drug solubilization and oral bioavailability have not previously been described (14).

In the current study, microemulsion preconcentrate formulations of the poorly water-soluble steroid, danazol [log P = 4.53 (15)] (Fig. 1), constructed using both long- and

[1] Department of Pharmaceutics, Victorian College of Pharmacy, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia.
[2] Department of Veterinary Sciences, The University of Melbourne, Werribee, Victoria 3030, Australia.
[3] Present address: Viikki Drug Discovery Technology Center, Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, Finland.
[4] To whom correspondence should be addressed. (e-mail: Chris.Porter@vcp.monash.edu.au)

0724-8741/04/0800-1405/0 © 2004 Plenum Publishing Corporation

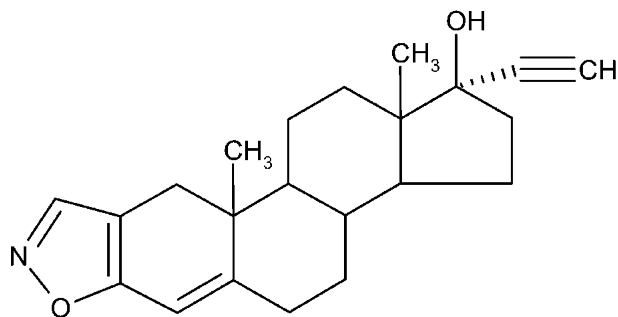

Fig. 1. Chemical structure of danazol.

medium-chain lipids, have been evaluated *in vitro* using both simple dispersion tests and the recently described lipid digestion protocol (16). The bioavailability of danazol after oral administration of the microemulsion preconcentrate formulations to beagle dogs has also been assessed and the *in vitro* and *in vivo* end-points compared. Danazol was also administered as a simple micronized powder formulation in both the fasted and postprandial states as a negative and positive control, respectively. The current data show the importance of appreciation of the impact of digestion on the solubilization capacity of lipidic formulations, including microemulsion formulations, *in vivo*, and provide further evidence for caution in the use of medium-chain lipids in digestible lipidic formulations.

MATERIALS AND METHODS

Materials

Danazol (pregna-2,4-dien-20-yno[2,3-d]isoxazol-17-ol) was kindly supplied by Sterling Pharmaceuticals (Sydney, Australia). Diazepam was from Alphapharm (Glebe, Sydney, Australia). Sodium taurodeoxycholate 99% (NaTDC) and porcine pancreatin (8× USP specifications activity) were from Sigma Chemical Co. (St. Louis, MO, USA). Captex 355 (MCT), a medium-chain triglyceride, and Capmul MCM, a blend of medium-chain mono-, di-, and tri-glycerides, were donated by Abitec Corporation (Janesville, WI, USA) and were used as received. Soybean oil (LCT), a long-chain triglyceride, was obtained from Sigma Chemical Co. Maisine 35-1, a blend of long-chain mono-, di-, and tri-glycerides, was a generous gift of Gattefossé (Saint-Priest, France). Lecithin [approximately 60% pure phosphatidylcholine (PC) by HPTLC (17) from egg yolk] was a gift from Pharmacia LKB (Uppsala, Sweden), 4-bromophenylboronic acid (4-BPB) was obtained from Aldrich Chemicals Co. (St. Louis, MO, USA) and 1 M sodium hydroxide (Titrisol), which was diluted with water to obtain 0.2 M NaOH titration solution, was purchased from Merck (Darmstadt, Germany). 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, respectively.

Preparation of Lipid-Based Formulations

The medium- and long-chain self-microemulsifying drug delivery system (MC-SMEDDS and LC-SMEDDS, respectively) formulations were similar to those previously described (8). Drug solubility in the two SMEDDS formulations was assessed using standard methodologies (3). Solubility was defined as the value attained when consecutive solubility samples varied by $\leq$5%. This was typically reached after equilibration times of between 48 and 72 h, and though this does not unequivocally preclude additional small (<5%) changes over longer time scales (and therefore may not represent an absolute equilibrium solubility), it represents a reasonable practical solubility estimation. Each unit dose of the medium-chain microemulsion preconcentrate contained danazol (5 mg), Captex 355 (360 mg), Capmul MCM (180 mg), Cremophor EL (360 mg), and absolute ethanol (100 mg). The corresponding long-chain microemulsion preconcentrate comprised danazol (5 mg), soybean oil (300 mg), Maisine 35-1 (300 mg), Cremophor EL (300 mg), and absolute ethanol (100 mg). The long-chain triglyceride solution formulation (LCT-solution) contained 5 mg danazol in 1 g of soybean oil (LCT). Lipidic formulations were prepared and individually filled into soft gelatin capsules by syringe as described previously (8). The micronized powder capsule formulation contained 15 mg danazol, 500 mg Avicel, and 500 mg Explotab filled into a single hard gelatin capsule.

In Vitro Evaluation

Dispersion Evaluation

The dispersion behavior of the LC-SMEDDS and MC-SMEDDS was evaluated by adding a soft gelatin capsule containing 1 g of either formulation to 200 ml of 0.1 N HCl maintained at 37°C in a USP II dissolution apparatus. Gentle agitation was provided by a stainless steel dissolution paddle mounted immediately below the solution meniscus and operated at 60 rpm. The dispersibility was monitored visually, and mean particle size of the resulting dispersion [determined by photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000] was assessed as described previously (8).

Drug Solubilization and Distribution Under Digesting Conditions

The *in vitro* digestion experiments were performed as previously described (11). Briefly, 250 mg of MC-SMEDDS or LC-SMEDDS formulation (containing 15 mg/g danazol) was dispersed in 9 ml of digestion buffer (50 mM TRIS maleate, 150 mM NaCl, 5 mM $CaCl_2 \cdot 2H_2O$, pH 7.5) containing 5 mM NaTDC and 1.25 mM PC [conditions broadly representative of fasted state intestinal conditions (18,19)]. Experiments were performed at 37°C in a stirred and thermostatted glass vessel and were initiated by the addition of 1 ml of pancreatin extract containing 10,000 tributyrin units (TBU) of pancreatic lipase (final lipase concentration 1000 TBU per ml). The digests were followed over 30 min using a pH-stat titration unit (Radiometer, Copenhagen, Denmark), which maintained the pH at 7.5. The fatty acids produced by lipolysis of the glycerides present in the vessel were titrated with 0.2 M NaOH. At the end of each experiment, a lipolysis inhibitor (4-BPB at 0.5 M in methanol, 9 μl/ml digestion medium) was added to the digestion medium to stop further digestion (14,20). Two 4-ml aliquots of the postdigestion mixtures were then taken from the digestion vessel and ultracentrifuged for 30 min at 37°C and 334000 g (Optima XL-100K centrifuge, SW-60 rotor, Beckman, Palo Alto, CA, USA) in

order to separate the digests into an aqueous dispersed phase and a pellet phase. Samples obtained from each separated phase were assayed for danazol content by HPLC as described below.

Drug Solubilization and Distribution Under Nondigesting Conditions

In addition to standard digestion studies, *in vitro* precipitation tests were also carried out under nondigesting conditions. In these studies, 250 mg of MC-SMEDDS or LC-SMEDDS (containing 15 mg/g danazol) were again dispersed in 9 ml of digestion buffer (50 mM TRIS maleate, 150 mM NaCl, 5 mM $CaCl_2 \cdot 2H_2O$, pH 7.5), containing 5 mM NaTDC and 1.25 mM PC at 37°C, in a stirred and thermostatted glass vessel. However in this case, pancreatic enzymes were not added. Systems were allowed to stir for 30 min in order to assess for the potential for danazol to precipitate due to dilution and dispersion (but not digestion) of the formulation. After 30 min, two 4-ml aliquots of the stirred mixtures were again ultracentrifuged for 30 min at 37°C and 334,000 × *g* (Optima XL-100K centrifuge, SW-60 rotor, Beckman) in order to separate the digests into a dispersed aqueous phase and a pellet phase. Samples obtained from each phase were assayed for danazol content by HPLC as described below.

Bioavailability Studies

All experimental procedures were approved and performed in accordance with the guidelines of the local Institutional Animal Experimentation Ethics Committee. The study was conducted as a five-way crossover (7-day washout) in four male beagle dogs (13.0–16.8 kg). There were five different treatments, three involving the fasted oral administration of 15 mg of danazol in lipid-based formulations, the fourth treatment was the fasted oral administration of 15 mg of danazol as a micronized powder, and the fifth treatment was the postprandial oral administration of 15 mg of danazol as a micronized powder. The treatments involving lipid-based formulations consisted of (i) three soft gelatin capsules containing danazol as a simple lipid solution in soybean oil (LCT). Total dose was 3 g of lipid and 15 mg of danazol (5 mg/g). (ii) Three soft gelatin capsules containing in total 3 g of MC-SMEDDS formulation and 15 mg of danazol (5 mg/g). (iii) Three soft gelatin capsules containing in total 3 g of LC-SMEDDS formulation and 15 mg of danazol. The micronized powder dose forms both consisted of 1 hard gelatin capsule containing 15 mg danazol, 500 mg Avicel, and 500 mg Explotab. With the exception of postprandial treatment with the micronized powder, the dogs were fasted for at least 20 h prior to each study day and the treatment was administered in the fasted state. The postprandial administration was accomplished by feeding a standard can of commercial dog food (680 g) containing 5% crude fat (max) 30–45 min prior to drug administration. After dosing, animals remained fasted until 10 h post-dose, after which they were fed on a daily basis. Each oral treatment was administered with 50 ml of water, which was otherwise available *ad libitum*. Blood samples (2.5 ml), obtained from the cephalic vein either via an indwelling catheter or by individual venipuncture were collected pre-dose (−10 min) and at 0.25, 0.5, 1, 2, 2.5, 3, 4, 6, 8, 10, 24, 32, 48, and 72-h post-dosing into sterile tubes containing 4.5 mg of dipotassium EDTA. Plasma was separated by centrifugation and stored (−20°C) until danazol analysis was performed using a validated HPLC assay.

Danazol Chromatography

Chromatography was conducted using a Beckman 126 Programmable Solvent Module, and Beckman 166 Programmable detector (Beckman Coulter, Fullerton, CA, USA) and a Waters 717 autosampler (Millipore). A Waters Symmetry C18 column (3.9 × 150 mm) (Millipore) was maintained at 45°C using a Waters 038040 Column Heater Module (Millipore). The mobile phase consisted of a mixture of solvent A (10% acetonitrile: 90% milli-Q water) and solvent B (90% acetonitrile: 10% milli-Q). The flow rate was 1.2 ml/min. The initial percentage of solvent B was 40%. This was increased to 50% over the first 2 min and was held at 50% solvent B for a further 13 min. After 15 min, the proportion of solvent B was increased to 90% over 5 min and then held at 90% until 24 min. At this time, the gradient returned to 40% solvent B over 1 min. Total run-time was 30 min. All samples were maintained at 10°C in the autosampler prior to injection of a 50-μl aliquot. Detection of danazol and the internal standard was conducted by single wavelength monitoring at 292 nm.

Sample Preparation

Calibration standards for danazol were prepared by spiking 1.0 ml aliquots of blank dog plasma with 100 μl of a 60:40 acetonitrile:water solution containing 0.05, 0.10, 0.50, 1.0, and 2.5 μg/ml danazol. This provided plasma standards in the concentration range 5–250 ng/ml danazol. One hundred microliters of 60:40 acetonitrile:water was similarly added to the plasma samples obtained during the pharmacokinetic studies to compensate for the volume of the spiking solution added to plasma standards. One hundred microliters of an internal standard solution (10 μg/ml of diazepam prepared in 60:40 acetonitrile:water) was also added to each plasma sample or standard, the tubes vortexed for 1 min, and 6 ml *n*-hexane added. The tubes were then vortexed for 2 min prior to centrifugation for 5 min at 3000 rpm in a Beckman GS-6R refrigerated centrifuge. The organic phase was transferred into new tubes and the contents evaporated to dryness under nitrogen at approximately 30°C using an N-EVAP evaporator (Organomation, Berlin, MA, USA). The dried extracts were reconstituted with 100 μl of a 60:40 acetonitrile:water solution, vortexed at high speed for 2 min, and centrifuged again (5 min at 3000 rpm, Beckman GS-6R). The entire volume of the reconstituted material (100 μl) was transferred to limited volume autosampler vials and 50 μl injected onto the HPLC.

Unknown concentrations were determined by comparison of the unknown danazol:IS peak height ratio with the calibration curve of danazol:IS peak height ratio vs. concentration constructed using the calibration standards.

Assay Validation

The plasma assay was validated by analysis of n = 5 quality control samples containing 5, 50, and 250 ng/ml danazol in blank plasma. The assay was found to be accurate to 92.4%, 92.8%, and 91.2% and precise to ± 13.0%, 3.1%, and 12.1% of 5 ng/ml, 50 ng/ml, and 250 ng/ml, respectively.

Pharmacokinetic Data Analysis

The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual plasma concentration vs. time profiles. The area under the plasma concentration vs. time profiles (AUC^{0-10}) was calculated by the linear trapezoidal method. Because the danazol plasma concentrations were typically below the limit of quantification of the assay at 24 and 26 h post-dose, accurate determination of the terminal elimination rate constant and ($AUC^{0-\infty}$) was not possible. However, because the plasma danazol concentrations at 10 h were low (<10 ng/ml), the extrapolated ($AUC^{10-\infty}$) was expected to contribute only a minor proportion of the ($AUC^{0-\infty}$), and consequently relative bioavailability comparisons were performed using AUC^{0-10}.

RESULTS

Formulation

The solubility of danazol in the MC-SMEDDS and LC-SMEDDS precursor solutions was 26.5 ± 0.7 mg/g and 16.1 ± 1.1 mg/g, respectively, at 37°C (mean ± SD, n = 3). Consequently, the preparation of the precursor solutions containing 15 mg/g resulted in clear isotropic solutions, which were subsequently loaded into soft gelatin capsules.

In Vitro Characterization

Dispersion and Particle Size

Dispersion of the SMEDDS formulations in 200 ml of 0.1 N HCl using the USP II dissolution apparatus resulted in an emulsion-like dispersion with a bluish tinge for both formulations, characteristic of a sub-micrometer particle size dispersion. The particle size of the predominant species was approximately 40 nm for both systems. The PCS data are presented in Table I, and reveal that for the MC-SMEDDS dispersion two populations were evident, the major population (~75%) being approximately 40 nm in diameter, whereas the minor population was approximately 125 nm in diameter. Similarly, for the LC-SMEDDS, populations of approximately 40 nm and 180 nm were detected, however an additional population of minor proportion with a particle size of approximately 1 μm was also seen, the presence of which resulted in an increase in the polydispersity index for the particle size distribution of the dispersed LC-SMEDDS formulation.

Table I. PCS Determination of Particle Size Distributions of the Dispersed SMEDDS Formulations

	Major population	Minor populations	Polydispersity
MC-SMEDDS	41.9 ± 1.6 nm	123.8 ± 3.9 nm	0.374 ± 0.013
LC-SMEDDS	40.4 ± 3.2 nm	(i) 180.5 ± 23.9 nm	0.812 ± 0.104
		(ii) 947 ± 166 nm	

Values are mean ± SD of three separate determinations for each formulation. Each determination consisted of three runs each of 10 sub-runs and were analyzed using the Contin method. PCS, photon correlation spectroscopy; MC, medium chain; LC, long chain; SMEDDS, self-microemulsifying drug delivery system.

Table II. Comparison of the Solubilization Profiles for MC-SMEDDS and LC-SMEDDS Systems Under Digesting and Nondigesting Conditions

	MC-SMEDDS	LC-SMEDDS
Nondigesting conditions		
Final aqueous phase conc.	331.7 μg/ml	361.6 μg/ml
% Aqueous phase	88.4	96.4
% Pellet	11.6	3.6
Digesting conditions		
Final aqueous phase conc.	116.1 μg/ml	351.6 μg/ml
% Aqueous phase	31	94
% Pellet	69	6

Two hundred fifty milligrams of formulation, containing 15 mg/g danazol was introduced into the *in vitro* digestion apparatus and either stirred for 30 min (nondigesting conditions) or dispersed for 10 min and then pancreatin added to initiate digestion and the sample digested for a further 30 min. Samples were subsequently ultracentrifuged to separate into an aqueous phase and a pellet phase in order to probe for drug precipitation. MC, medium chain; LC, long chain; SMEDDS, self-microemulsifying drug delivery system.

In Vitro Digestion Studies

In contrast to the simple dispersion studies, *in vitro* digestion of the microemulsion formulations revealed markedly different drug distribution patterns for the MC-SMEDDS and LC-SMEDDS (Table II). Under standard digesting conditions, fatty acid titration had concluded prior to 30 min in all cases, indicating that digestion was complete. No residual oil phase was apparent on ultracentrifugation of the digestion media. Analysis of drug content in the aqueous and pellet phases separated by ultracentrifugation revealed that the MC-SMEDDS formulation retained only 31% of the drug in the aqueous phase after 30 min digestion, with the remaining 69% being recovered in the precipitated pellet. In contrast, the LC-SMEDDS formulation retained 94% of drug in the aqueous phase after the 30-min digestion period, and only 6% of drug was recovered in the pellet (Table II).

Danazol Solubilization Under Nondigesting Conditions

In contrast to the data obtained under digesting conditions, simply stirring the MC-SMEDDS and LC-SMEDDS formulations in digestion buffer for 30 min resulted in only minor levels of precipitation for both formulations (11.6% and 3.6% respectively, Table II).

Bioavailability Data

The mean plasma concentration vs. time profiles for danazol following oral administration of the LC-SMEDDS, MC-SMEDDS, or LCT lipid solution formulations are shown in Fig. 2A. To aid comparison of C_{max} and T_{max}, truncated data to 10 h are presented in Fig. 2B. In the interests of clarity, the plasma levels obtained after fasted and postprandial administration of danazol as a simple micronized powder formulation (Fig. 2C) are presented separately to the data for the lipid-based formulations (data truncated to 10 h in Fig. 2D). The corresponding mean pharmacokinetic parameters for all dose forms, after normalizing to a standard 15 mg dose (to circumvent small differences in fill weights for individual capsules), are tabulated in Table III.

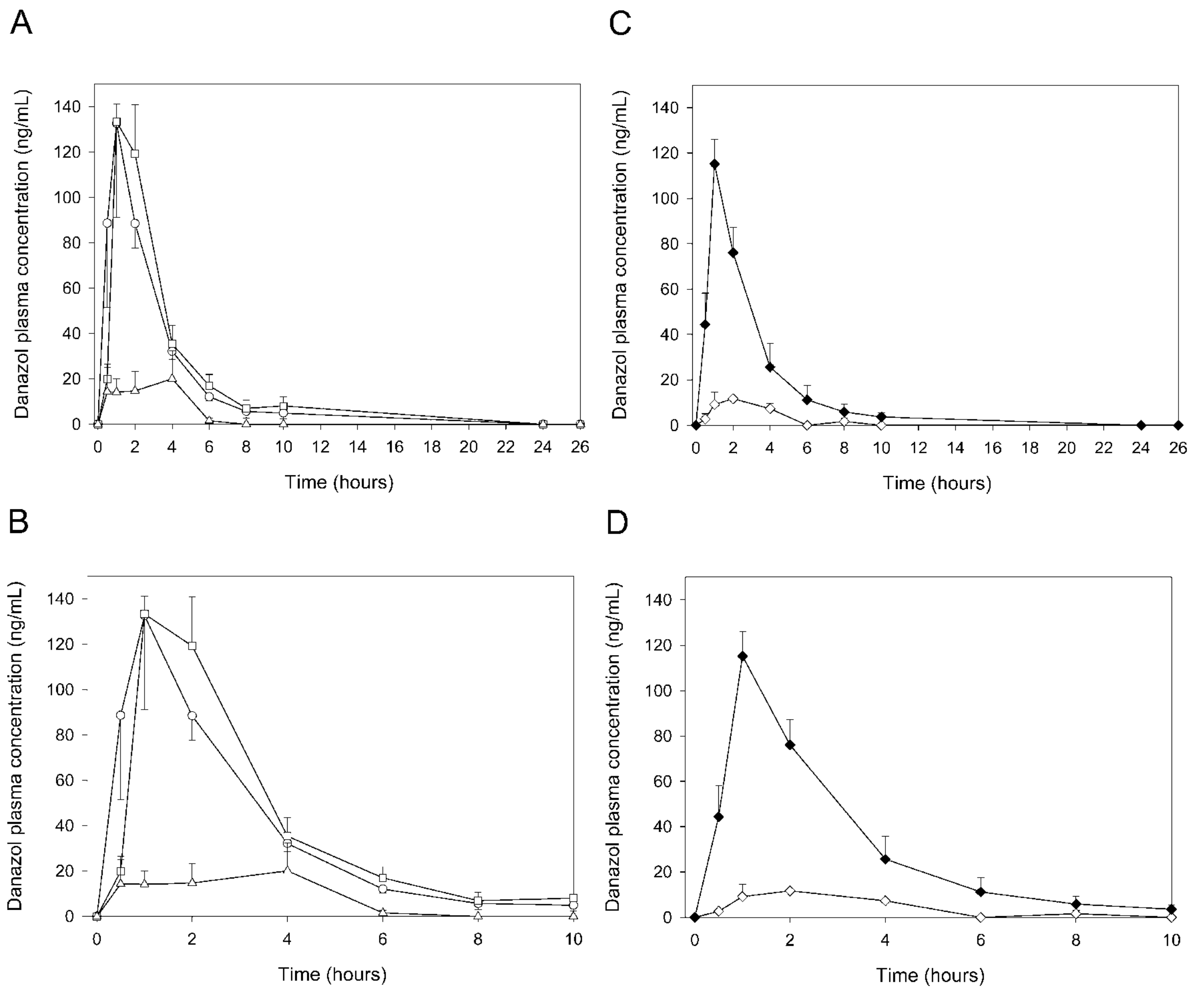

Fig. 2. Mean plasma concentration vs. time profiles (mean ± SE, n = 4) for danazol following oral administration to beagle dogs. A total dose of 15 mg danazol was administered for each treatment. Three treatments involved administration in the fasted state as lipidic solutions containing danazol as a 5 mg/g solution (3 × soft gelatin capsules) in (△) MC-SMEDDS, (○) LC-SMEDDS, or (□) LCT-solution; the resulting plasma profiles obtained over the entire sampling period are presented in (A), and data truncated to 10 h to facilitate easier comparison of C_{max} and T_{max} are presented in (B). Plasma profiles, after two further treatments with a dry micronized powder form of danazol (15 mg in one hard gelatine capsule), administered in the fasted (◊) and post-prandial (♦) states, are presented in (C), with truncated data in (D). (MC-SMEDDS, medium-chain triglyceride-based self-microemulsifying drug delivery system; LC-SMEDDS, long-chain triglyceride-based self-microemulsifying drug delivery system; LCT-solution, drug solution in simple triglyceride.)

Table III. Summary PK Parameters for Danazol after Oral Administration to Beagle Dogs as a Micronized Powder in the Fasted and Postprandial States or after Fasted Administration of Three Alternate Lipid-Based Formulations (Normalized to 15 mg Dose[a])

	Fasted administration				Postprandial administration
	Micronized powder	LCT lipid solution	LC-SMEDDS	MC-SMEDDS	Micronized powder
C_{max} (ng/ml)	16.0 ± 3.6	154.9 ± 34.1	96.2 ± 4.0	26.3 ± 10.9	117.5 ± 11.1
T_{max} (h)	2.6 ± 0.7	1.1 ± 0.3	1.0 ± 0.1	1.9 ± 0.9	1.2 ± 0.1
Elimination $t_{1/2}$ (h)	NA	1.6 ± 0.5	1.6 ± 0.6	NA	1.5 ± 0.5
$AUC^{0\text{-}10}$ (ng · h/ml)	35.3 ± 5.2	340.2 ± 64.4	270.5 ± 38.5	47.7 ± 25.9	265.1 ± 33.5
Rel BA[b] (%)	100	963 ± 187	766 ± 109	135 ± 76	751 ± 95

PK, pharmacokinetic; LCT, long-chain triglyceride; LC, long chain; MC, medium chain; SMEDDS, self-microemulsifying drug delivery system.

[a] The actual dose administered varied across groups due to changes in capsule fill weights and concentrations. The mean dose was 15.3 ± 3.2 mg (mean ± SD; n = 20)

[b] Rel BA is the relative bioavailability (%) in comparison to fasted administration of the danazol powder formulation as determined by the ratio of the dose-normalized $AUC^{0\text{-}10}$ data.

The terminal half-lives ($t_{1/2}$) for all treatments (with the exception of the MC SMEDDS and fasted micronized powder formulations, where the low plasma levels precluded accurate $t_{1/2}$ assessment) were consistent and in the range reported previously for beagle dogs [2.7 ± 1.4 h (21)]. A clear distinction in terms of relative bioavailability and C_{max} was seen between the lipid-based dose forms, where the bioavailability relative to the powder formulation was considerably higher for the LCT-solution (963 ± 187%) and LC-SMEDDS (766 ± 109%) when compared to the MC-SMEDDS (135 ± 76%). Similarly, the C_{max} values for the LCT-solution (154.9 ± 34.1 ng/ml), and the LC-SMEDDS (96.2 ± 4.0 ng/ml) were significantly higher ($p < 0.02$) than the MC-SMEDDS formulation (26.3 ± 10.9 ng/ml).

As expected, postprandial administration of the powder provided a large increase in relative bioavailability (751 ± 95%) and C_{max} (117.5 ± 11.1 ng/ml) when compared to the fasted administration of the powder (100% nominal bioavailability, C_{max} = 16.0 ± 3.6 ng/ml). Importantly, the C_{max} and relative bioavailability for the long-chain lipid formulations (LCT-solution and LC-SMEDDS) were statistically indistinguishable from that of the powder formulation after postprandial administration to fasted dogs. This suggests that appropriate lipidic formulations may be capable of achieving the same positive food effect as postprandial administration.

DISCUSSION

Lipid formulations in general, and self-emulsifying and self-microemulsifying systems in particular, are a popular means by which to increase the oral bioavailability of poorly water-soluble drugs. However, some lipidic formulations appear to perform markedly better than others, and a common rationale by which these differences may be explained is still not clear.

The current studies were conducted primarily to compare the *in vivo* performance of two self-microemulsifying drug delivery systems containing either medium chain triglyceride (in the case of MC-SMEDDS) or long-chain triglyceride (LC-SMEDDS), with that of a simple long-chain triglyceride solution formulation (LCT-solution), and to evaluate the utility of different *in vitro* methodologies to predict the *in vivo* formulation differences. Danazol was chosen as a model poorly water-soluble drug. Additionally, a micronized powder formulation, similar to the commercial Danocrine capsule formulation of danazol, was also administered in the fed and fasted states. (Danocrine contains lactose, starch-maize, magnesium stearate, purified talc, and titanium dioxide, whereas the formulation used here utilized a simple dry blend of danazol with Avicel and Explotab). The results of these *in vivo* studies, and the supporting *in vitro* dispersion and digestion data, have allowed comparison of the effects of lipid type on formulation digestion and drug disposition in the gastrointestinal milieu and the impact of these processes on subsequent drug absorption.

Effect of Lipid Chain Length on *in Vitro* and *in Vivo* Performance of Microemulsion Formulations

The conventional *in vitro* indicators of likely microemulsion formulation performance, namely the capacity to dissolve lipophilic drugs in the formulation and the ability to disperse the formulation rapidly to form small colloidal particles, were extremely positive for both the medium-chain and long-chain SMEDDS formulations and the isotropic MC-SMEDDS and LC-SMEDDS precursor solutions dispersed rapidly on dilution to form fine microemulsion-like sub-micrometer dispersions (Table I). Furthermore, on stirring in the simulated fasted intestinal fluid for 30 min at 37°C under nondigesting conditions, little precipitation was evident in either dispersed formulation (Table II).

In contrast to these positive *in vitro* indicators, the plasma profiles in Fig. 2 and the pharmacokinetic parameters presented in Table III reveal that the MC-SMEDDS system performed poorly *in vivo* when compared to the LC-SMEDDS system. Administration of the MC-SMEDDS resulted in 5-fold lower relative bioavailability of danazol when compared with that obtained after fasted administration of the LC-SMEDDS formulation. A delayed and variable T_{max} and C_{max} was also apparent when compared with the LC-SMEDDS and LCT-solution formulations.

Comparison of the data obtained with the lipidic formulations obtained in this study with those obtained after administration of a micronized powder dose form in the fed and fasted states also allows some indication of lipid formulation performance relative to the likely best and worst-case scenarios, respectively, for absorption of the same dose of danazol. Thus, postprandial administration of a micronized powder formulation (where the large amount of lipid typically present in a meal (~30 g) is co-administered with the drug) is recognised as giving an indication of the maximum likely extent of absorption from a lipidic formulation, whereas fasted state administration of a simple powder formulation might be expected to provide the poorest absorption characteristics. Comparison of the pharmacokinetic parameters in Table III suggest that the MC-SMEDDS system provides little benefit over the lipid-free micronized powder even though using simple *in vitro* dispersion testing, the formulation attributes (a well dispersed, lipid-based dose form with high solubilizing power for the drug) appeared positive. In contrast, both long-chain triglyceride-based dose forms resulted in a similar level of danazol exposure to that seen after postprandial administration.

The apparent inconsistency between *in vitro* and *in vivo* end-points may be explained by the behavior of the lipidic dispersions on digestion. *In vitro* digestion studies conducted with the LC-SMEDDS formulation revealed efficient transfer of 94% of danazol into the aqueous phase of the digest in a solubilized form and resulted in virtually no precipitation of drug on digestion of the lipids present in the formulation. In dramatic contrast, however, *in vitro* digestion of the MC-SMEDDS system resulted in 70% of the drug being lost to the pellet phase, indicating that significant drug precipitation had occurred.

We have previously shown that the mass of lipid used in *in vitro* digestion studies of medium chain triglyceride-based formulation can be critical in determining the type of colloidal structures that are present in the resultant aqueous phase (13). Specifically, we have shown that *in vitro* digestion of large masses of medium-chain lipid (250 mg) in the same *in vitro* digestion protocol as that used in these studies may result in the formation of a vesicular phase that has high solubilizing capacity for lipophilic drugs. In contrast, the *in vitro* digestion of lesser amounts of lipid (125 mg and 50 mg)

typically resulted in the production of a micellar system with a relatively lower capacity for drug solubilization. In the current *in vitro* studies 135 mg MCT was used [54% (w/w) of the MC-SMEDDS formulation]. It is likely therefore that digestion of this formulation resulted in the formation of mixed micelles containing exogenous surfactant, lipid digestion products, and the endogenous lipids (bile salts and phospholipids) present in the digestion medium. We have also previously shown that at molar equivalent fatty acid content, medium-chain mixed micelles are an order of magnitude less effective at solubilizing danazol than long-chain mixed micelles (3), and this provides a possible explanation for the reduced capacity of the digested medium-chain formulation to solubilize danazol (and facilitate absorption) when compared to the digested long-chain formulation.

Interestingly, this outcome is somewhat counter-intuitive when considering the relatively large quantity of surfactant present in both SMEDDS formulations (Cremophor constitutes 36% w/w of the MC-SMEDDS and 30% w/w of the LC-SMEDDS precursor solutions) and which might be expected to facilitate drug solubilization after digestion of both formulations. It appears therefore that although surfactants such as Cremophor are commonly used to solubilize poorly-water soluble drugs in aqueous media, in these studies with danazol the surfactant appears to provide little solubilization enhancement over that provided by the lipids alone. In particular, the presence of the Cremophor in the MC-SMEDDS formulation does little to improve its relative *in vivo* performance above that of the dry powder.

Previous studies of the *in vitro* and *in vivo* behavior of the poorly water-soluble anti-malarial halofantrine (aqueous solubility = <0.1 μg/g, log P = 8.86) in surfactant-free LCT and MCT-solutions (11,13), using techniques similar to those described here, have shown similar trends. Thus the relative oral bioavailability of halofantrine after administration of a LCT-solution formulation to beagle dogs was 5-fold greater that that from a corresponding MCT-solution formulation over a 10-h post-dosing period. In concurrent *in vitro* digestion studies, halofantrine was also observed to precipitate extensively on digestion of the MCT lipid solution (>90% precipitated) when compared to the LCT solution (22% precipitated) (13). This behavior is in agreement with the current findings and suggests that MCT-based formulations, regardless of the presence of additional surfactant, such as that contained in the microemulsion precursor formulations of the current study, when subjected to digestion processes, have a reduced capacity to solubilize some lipophilic drugs when compared to LCT-based formulations, and may limit the extent of oral absorption.

Effect of Formulation Type on *in Vitro* and *in Vivo* Performance of LCT-Based Formulations

Though the *in vivo* behavior of the MC-SMEDDS closely resembled that of the dry powder dose form, absorption of danazol after administration of the highly dispersed LC-SMEDDS formulation was surprisingly no better than that of the LCT-solution. However, the digestion of LCT to form long-chain fatty acids and monoglycerides, and their intercalation into bile salt/phospholipid mixed micelles, to provide a highly dispersed intestinal milieu with high solubilizing capacity, is a well-characterized process (22,23). It appears likely therefore that the digestion products of the LCT formulation form similar mixed micellar systems with the bile salts and phospholipids present *in vivo*, to that provided by SBO and Maisine in the LC-SMEDDS, and essentially provide the same postdigestion, preabsorption intestinal milieu as the LCT-solution system. It further appears that the presence of Cremophor, or the dispersion of the dose form to provide a microemulsion, imparts no additional bioavailability benefit above that of a simple lipid solution formulation.

These results suggest, therefore, that the *in vivo* performance of lipid-based formulations may be more sensitive to the physicochemical properties of the lipid digestion products rather than the nature of the formulation. In the case of digestible lipid-based formulations, digestion results in the loss of the initial physical form of the formulation, and it is likely that the solubilizing power of the colloidal phases that result dictates the pattern of drug absorption far more avidly than the physical state of the initial formulation.

Finally, the adage that "smaller is better" has been responsible to a large degree for the use of self-emulsifying and self-microemulsifying systems in an attempt to improve bioavailability of poorly water-soluble drugs. In turn, the addition of relatively large amounts of surfactants to facilitate rapid and effective *in vitro* dispersion of formulations has been used to provide for this perceived improvement in formulation performance. However, the current results suggest that in some situations, the surfactant (in this case Cremophor), while facilitating dispersion, may not provide any additional improvement in drug absorption. [As a caveat, it should be acknowledged that surfactants may have beneficial properties for specific compounds under certain circumstances, for example via inhibition of drug efflux (24)].

In conclusion, the data obtained in these studies illustrate a number of design concepts that might usefully be incorporated into formulation strategies for lipid-based formulations of poorly water-soluble drugs. Thus, approaches to the design of lipidic formulations based on drug solubility in the dose form and dispersibility of the formulation should be used with caution—an elegant formulation with high solubility for the drug and exceptional dispersion properties, such as the MC-SMEDDS in the current study, may not lead to a satisfactory level of *in vivo* performance. In contrast, consideration of the likely colloidal species formed on digestion of a lipid system appears to be a more powerful indicator of *in vivo* performance. The different capacities of micellar species to solubilize lipophilic compounds (a property that is primarily dictated by the nature of their lipid constituents) appears to be an important determinant of the ultimate performance of the formulation *in vivo*. Furthermore, the similar *in vivo* danazol exposure obtained with the seemingly dissimilar LCT-solution and LC-SMEDDS systems suggests that two seemingly different formulations may, on digestion, present the intestine with essentially the same preabsorption medium and lead to a similar extent of absorption.

ACKNOWLEDGMENTS

Partial funding for AMK from the Finnish Academy, the Finnish Cultural Foundation, and the University of Helsinki, Finland, is gratefully acknowledged. We also thank Ms. Jacqueline O'Connor for excellent technical assistance.

REFERENCES

1. A. J. Humberstone and W. N. Charman. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Adv. Drug Deliv. Rev.* **25**:103–128 (1997).
2. K. J. MacGregor, J. K. Embleton, J. E. Lacey, E. A. Perry, L. J. Solomon, H. Seager, and C. W. Pouton. Influence of lipolysis on drug absorption from the gastrointestinal tract. *Adv. Drug Deliv. Rev.* **25**:33–46 (1997).
3. G. K. Kossena, B. J. Boyd, C. J. H. Porter, and W. N. Charman. Separation and characterisation of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water soluble drugs. *J. Pharm. Sci.* **92**:634–648 (2003).
4. M. Lindström, H. Ljusberg-Wahren, K. Larsson, and B. Borgström. Aqueous lipid phases of relevance to intestinal fat digestion and absorption. *Lipids* **16**:749–754 (1981).
5. P. P. Constantinides. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharm. Res.* **12**:1561–1572 (1995).
6. T. Gershanik and S. Benita. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. *Eur. J. Pharm. Biopharm.* **50**:179–188 (2000).
7. R. M. Klauser, H. Irshik, J. Kletzmayr, I. Sturm, W. Brunner, W. Woloszczuk, and J. Kovarik. Pharmacokinetic cyclosporine A profiles under long-term Neoral treatment in renal transplant recipients: does fat intake still matter? *Transplant. Proc.* **29**:3137–3140 (1997).
8. S. M. Khoo, A. J. Humberstone, C. J. H. Porter, G. A. Edwards, and W. N. Charman. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. *Int. J. Pharm.* **167**:155–164 (1998).
9. D. J. Hauss, S. E. Fogal, J. V. Ficorilli, C. A. Price, T. Roy, A. A. Jayaraj, and J. J. Keirns. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor. *J. Pharm. Sci.* **87**:164–169 (1998).
10. C. W. Pouton. Formulation of self-emulsifying drug delivery systems. *Adv. Drug Deliv. Rev.* **25**:47–58 (1997).
11. A. M. Kaukonen, B. J. Boyd, C. J. H. Porter, and W. N. Charman. Drug solubilization behaviour during *in vitro* digestion of simple triglyceride lipid solution formulations. *Pharm. Res.* **21**: 245–253 (2004).
12. A. M. Kaukonen, B. J. Boyd, W. N. Charman, and C. J. H. Porter. Drug solubilization behaviour during *in vitro* digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. *Pharm. Res.* **21**:254–260 (2004).
13. C. J. H. Porter, A. M. Kaukonen, A. Taillardat-Bertschinger, B. J. Boyd, J. M. O'Connor, G. A. Edwards, and W. N. Charman. Use of *in vitro* lipid digestion data to explain the *in vivo* performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: studies with halofantrine. *J. Pharm. Sci.* **93**: 1110–1121 (2004).
14. J. P. Reymond and H. Sucker. *In vitro* model for ciclosporin intestinal absorption in lipid vehicles. *Pharm. Res.* **5**:673–676 (1988).
15. A. Taillardat-Bertschinger, C. A. M. Martinet, P. A. Carrupt, M. Reist, G. Caron, R. Fruttero, and B. Testa. Molecular factors influencing retention on immobilized artifical membranes (IAM) compared to partitioning in liposomes and n-octanol. *Pharm. Res.* **19**:729–737 (2002).
16. L. Sek, C. J. H. Porter, A. M. Kaukonen, and W. N. Charman. Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. *J. Pharm. Pharmacol.* **54**:29–41 (2002).
17. L. Sek, C. J. H. Porter, and W. N. Charman. Characterisation and quantification of medium chain and long chain triglycerides and their in vitro digestion products, by HPTLC coupled with *in situ* densitometric analysis. *J. Pharm. Biomed. Anal.* **25**:651–661 (2001).
18. D. W. Hay, M. J. Cahalane, N. Timofeyeva, and M. C. Carey. Molecular species of lecithins in human gallbladder bile. *J. Lipid Res.* **34**:759–768 (1993).
19. W. C. Duane, R. L. Ginsberg, and L. J. Bennion. Effects of fasting on bile acid metabolism and biliary lipid composition in man. *J. Lipid Res.* **17**:211–219 (1976).
20. J. E. Staggers, O. Hernell, R. J. Stafford, and M. C. Carey. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 1. Phase behavior and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. *Biochemistry* **29**:2028–2040 (1990).
21. L. Erlich, D. Yu, D. A. Pallister, R. S. Levinson, D. G. Gole, P. A. Wilkinson, R. E. Erlich, L. E. Reeve, and T. X. Viegas. Relative bioavailability of danazol in dogs from liquid-filled hard gelatin capsules. *Int. J. Pharm.* **179**:49–53 (1999).
22. M. C. Carey, D. M. Small, and C. M. Bliss. Lipid digestion and absorption. *Annu. Rev. Physiol.* **45**:651–677 (1983).
23. J. E. Staggers, O. Hernell, R. J. Stafford, and M. C. Carey. Physical-chemical behaviour of dietary and biliary lipids during intestinal digestion and absorption. 1. Phase behaviour and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. *Biochemistry* **29**: 2028–2040 (1990).
24. M. M. Nerurkar, P. S. Burton, and R. T. Borchardt. The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. *Pharm. Res.* **13**:528–534 (1996).