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A lipid-based liquid crystalline matrix that provides sustained release and enhanced oral bioavailability for a model poorly water soluble drug in rats

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Abstract

Liquid crystalline phases that are stable in excess water, formed using lipids such as glyceryl monooleate (GMO) and oleyl glycerate (OG), are known to provide a sustained release matrix for poorly water soluble drugs in vitro, yet there has been no report of the use of these materials to impart oral sustained release behaviour in vivo. In the first part of this study, in vitro lipolysis experiments were used to compare the digestibility of GMO with a second structurally related lipid, oleyl glycerate, which was found to be less susceptible to hydrolysis by pancreatic lipase than GMO. Subsequent oral bioavailability studies were conducted in rats, in which a model poorly water soluble drug, cinnarizine (CIN), was administered orally as an aqueous suspension, or as a solution in GMO or OG. In the first bioavailability study, plasma samples were taken over a 30 h period and CIN concentrations determined by HPLC. Plasma CIN concentrations after administration in the GMO formulation were only sustained for a few hours after administration while for the OG formulation, the plasma concentration of cinnarizine was at its highest level 30 h after dosing, and appeared to be increasing. A second study in which CIN was again administered in OG, and plasma samples taken for 120 h, revealed a T_{max} for CIN in rats of 36 h and a relative oral bioavailability of 344% when compared to the GMO formulation (117%) and the aqueous suspension formulation (assigned a nominal bioavailability of 100%). The results indicate that lipids that form liquid crystalline structures in excess water, may have application as an oral sustained release delivery system, providing they are not digested rapidly on administration.

Keywords: Poorly water soluble drug; Lipid formulation; Liquid crystal; Cinnarizine; Bioavailability

1. Introduction

There has been much recent interest in the use of self-assembled nanostructured materials for controlling the release of incorporated agents across a range of possible applications. In the pharmaceutical arena, viscous lipid-based systems, such as bicontinuous cubic and hexagonal liquid crystalline phases offer considerable scope for application as drug delivery systems (Drummond and Fong, 1999). They have the potential for control over release rates, low toxicity, and versatility in application across a range of administration regimes, including oral (Chung et al., 2002), transdermal (Chang and Bodmeier, 1994; Lopes et al., 2006) and parenteral delivery (Engstrom et al., 1996; Chang and Bodmeier, 1998; Boyd et al., 2006). Lipid-based liquid crystalline systems are also mucoadhesive (Norling et al., 1992; Nielsen and Hansen, 1998) and have consequently been used as the basis for a metronidazole periodontal gel product (Jones et al., 1999).

An important attribute of a limited number of lipid-based liquid crystalline systems is that they are thermodynamically stable in excess water, thereby providing a persistent matrix on exposure to liquids such as gastrointestinal (GI) and interstitial fluids. This property also allows for the pre-dispersion of liquid crystalline systems in aqueous vehicles in the form of sub-micron particles suitable for intravenous drug delivery

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(Gustafsson et al., 1997). Materials known to exhibit such phase behaviour include phospholipids, alkyl glycerides such as glyceryl monooleate (GMO) (Briggs and Caffrey, 1994), amphiphiles with phytanyl chains such as phytantriol (Barauskas and Landh, 2003) and glycolipids (Hato and Minamikawa, 1996), and alkyl glycerates (Boyd et al., 2006).

We have recently reported the ability of alkyl glycerates to sustain the release of both hydrophilic and hydrophobic drugs in vitro (Boyd et al., 2006). At physiological temperatures oleyl glycerate (OG), which has a very close structural relationship to GMO (Fig. 1), forms a reverse hexagonal phase in excess water, providing an interesting point of differentiation with GMO (which instead forms a bicontinuous cubic phase). The structural difference between reverse hexagonal phase and bicontinuous cubic phase resulted in slower release of the hydrophobic drugs, paclitaxel and irinotecan from the reverse hexagonal matrix formed by OG when compared to the release of the same materials from GMO-based cubic phase.

The ability of drug delivery systems based on lipids such as GMO to enhance the bioavailability of poorly water soluble drugs after oral administration, is well known, and is thought to reflect the ability of lipids and their digestion products to interact with endogenous bile-salt phospholipid micelles in the gastrointestinal tract (GIT), resulting in an increase in the solubilization capacity of the GI fluids (Charman et al., 1993). However, although GMO forms cubic phase in aqueous environments (Clogston et al., 2000), there is no literature evidence supporting the use of GMO for providing extended release of poorly water soluble drugs after oral administration. The ester bond of GMO is rapidly cleaved by pancreatic lipase during the digestion of dietary and formulation lipids (Sek et al., 2002) to form oleic acid and glycerol, and therefore the failure of GMO to provide a sustained release effect for orally administered drugs may be attributed to enzymatic breakdown of cubic phase in the digestive tract. The relative susceptibility of OG to lipolysis compared with GMO is unknown, but it seems likely that OG may be more resistant to lipolysis when compared to GMO, and therefore could provide a more persistent matrix for sustained release in the GIT.

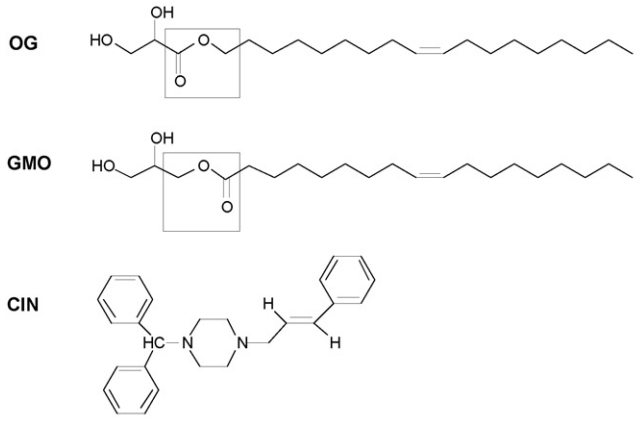

Fig. 1. Chemical structures of the lipids used in this study, oleyl glycerate (OG), glyceryl monooleate (GMO), and the model poorly water soluble drug, cinnarizine (CIN). The dashed boxes indicate the region of structural difference between the OG and GMO molecular structures.

Consequently, the current study was undertaken to examine the relative digestibility of GMO and OG using in vitro lipid digestion experiments. Differences between the digestibility of the lipids prompted subsequent in vivo bioavailability experiments to investigate whether these differences in digestibility may impact on the in vivo behaviour of lipid formulations based on OG and GMO, and in particular, to assess whether sustained release of a model poorly water soluble drug could be achieved using OG. Cinnarizine (CIN) was selected as a poorly water-soluble drug, and was dosed orally to rats in three formulations—a lipid solution in OG, a lipid solution in GMO and as an aqueous suspension. Studies were conducted with the expectation that the OG and GMO formulations would form reverse hexagonal phase and bicontinuous cubic phase, respectively, on exposure to excess fluids in the GI tract. Drug plasma concentrations were determined over time to elucidate the effect of the liquid crystalline matrix on drug absorption. Histopathological examination of intestinal tissues was also conducted to assess the potential effect of OG on these tissues, and to exclude the possibility that tissue damage may be responsible for differences in absorption behaviour that may result.

2. Methods and materials

2.1. Materials

The commercial material Myverol 18-99K (Kerry Bioscience, Almere, The Netherlands) was used in place of pure GMO as it has been shown previously that the phase behaviour of pure GMO and Myverol 18-99K are very similar, and importantly that both materials form cubic phase in excess water at physiological temperatures (Clogston et al., 2000). Myverol 18-99K comprises >90% unsaturated monoglycerides, including >60% glyceryl monooleate. OG was supplied by IDT, Boronia, Australia, and was >99% pure by liquid chromatography–mass spectrometry (LC–MS). Porcine pancreatin, Trizma maleate, Pluronic F127, cinnarizine, Tween 80, sodium carboxymethyl cellulose (medium viscosity), sodium hydroxide, sodium taurodeoxycholate (BS) and 2-monoolein were purchased from Sigma Chemical Co. (St. Louis, MO) and were of analytical purity. HCl solution was purchased from BDH chemicals (Poole, Dorset, UK). Phospholipid (PC) was from Pharmacia (Uppsala, Sweden), and was determined by high performance thin layer chromatography (HPTLC) to comprise approximately 60% phosphatidylcholine (Sek et al., 2002). Water was obtained from a MilliQ purification system (Millipore, Australia).

2.2. Phase behaviour by crossed polarised light microscopy

The phase behaviour of OG and GMO containing cinnarizine, when in contact with water and 0.1N HCl (as a model gastric fluid), was confirmed by crossed polarised light microscopy at 37 °C, using the penetration scan technique described previously (Boyd et al., 2006). A solution of cinnarizine in each lipid (25 mg CIN/g lipid) was prepared at 37 °C. A drop was

placed between a microscope slide and coverslip maintained at 37 °C using a microscope hot stage (HFS 91 and TP-93, Linkam, Surrey, England) and a drop of water or 0.1N HCl added to the edge of the coverslip to contact the interface with the lipid droplet. The appearance at the interface was then photographed when viewed through crossed polarised filters on a Zeiss Axiolab E microscope (Carl Zeiss, Melbourne Australia) fitted with a Canon Powershot digital camera.

2.3. In vitro digestion studies

In vitro digestion studies were conducted in digestion media designed to simulate fasted intestinal conditions (5 mM sodium taurodeoxycholate (BS) and 1.25 mM phosphatidylcholine (PC) in digestion buffer comprising 50 mM trizma maleate, 150 mM NaCl, and 5 mM $CaCl_2{\cdot}2H_2O$ at pH 7.5) and were conducted in a similar manner to those described by Sek et al. (2002). It was necessary to form coarse dispersions of GMO and OG prior to commencing digestion studies, as the viscous cubic phase and reverse hexagonal phases formed on addition of digestion buffer to GMO and OG prevented adequate stirring and adhered to the electrode and burette in the digestion vessel. To facilitate formulation dispersion a solution of Pluronic F127 (10%, w/w) in 2 g of OG or GMO was prepared at 50 °C by vortex mixing. The lipid + Pluronic mixtures were then added drop wise to 18 mL of digestion buffer at 50 °C under shear provided by an Ultraturrax homogenizer (T25 fitted with S25N-8G dispersing element from IKA, Staufen, Germany) at 16,000 rpm over 30 s. Dispersions were then homogenized for an additional 60 s, and allowed to cool to room temperature over 30 min. 2 mL of the coarse dispersion was added to 16 mL of simulated fasted intestinal medium, and the pH was adjusted to 7.500 ± 0.005, prior to addition of 20,000 TBU equivalents of porcine pancreatin in 2 mL of digestion buffer. Addition of the enzyme stimulated digestion of the lipid formulation components, and the progress of digestion was followed using a pH stat (Radiometer Pacific, Australia) by monitoring the volume of titrant (0.6 M NaOH) required to maintain the pH at 7.500. Titration profiles for the blank, lipid free medium were subtracted from the OG and GMO profiles to obtain the digestion profiles of the lipids alone.

To enable determination of lipid concentration in the digestion medium over time, at set time points during digestion (0, 5, 10, 20, 30, 45 and 60 min) a 0.1 mL aliquot was removed from the digestion vessel, and immediately diluted in 0.9 mL acetonitrile to quench the digestion process. The concentration of GMO and OG in the in vitro digestion samples was determined by HPTLC and HPLC-UV, respectively. The HPTLC method for the determination of GMO has been reported previously (Sek et al., 2001). Briefly, the digestion samples were further diluted in an acetonitrile:ethanol 2:1 (v/v) solution and a standard curve was constructed using 2-monoolein as an analytical standard from 0.2 to 2 μg on the plate. The samples and standards were separated on HPTLC plates (20 cm × 10 cm Silica gel 60 F254) by elution with a series of solvents on an automated multiple development chamber (AMD 2, Camag, Mullentz, Switzerland), and the plates developed using $CuSO_4{\cdot}5H_2O$–H_3PO_4 (85%, w/w)-Milli-Q water (10:8:82, w/v/v) solution as described previously (Sek et al., 2001). Densitometry was then used to quantify the amount of GMO in each sample. For determination of OG in the digestion samples, the HPLC system comprised a Waters 717 Autosampler, Beckman 126 solvent module, a Beckman 168 photo diode array UV detector and were analysed using Beckman Nouveau Gold software. Samples were eluted on a Beckman Ultrasphere C_8 column (250 mm × 4.6 mm, 5 μm) (Phenomenex, Sydney, Australia) at 1 mL/min, using a mobile phase comprising acetonitrile/MilliQ water (90/10, v/v), with a 20 μL injection volume and UV detection at 200 nm. The retention time of OG under these conditions was 6.8 min. A standard curve was constructed between 1 and 10 μg/mL, and samples were diluted into this range in acetonitrile/MilliQ water (90/10, v/v) as required.

2.4. Oral bioavailability studies

2.4.1. Study design

All experimentation involving animals for this project was approved by the Victorian College of Pharmacy Animal Ethics Committee, Monash University, Parkville, Vic., Australia.

Two separate in vivo studies in rats were conducted in which the oral absorption of the model lipophilic drug, cinnarizine (CIN), was investigated after oral administration of formulations based on GMO, OG and a control suspension formulation. In the first study (Study 1), data was obtained for a period of 30 h after administration of the different formulations. The results of this initial study, however, indicated that a second study (Study 2) of longer duration was required to fully capture the pharmacokinetic profile of cinnarizine from the OG formulation, and therefore for this second study, samples were obtained for up to 120 h after administration of the OG formulation.

2.4.2. Animal procedures

On the day prior to dosing, a cannula (polyethylene tubing, 0.96 mm × 0.58 mm, Paton Scientific, Victor Harbour, Australia) was surgically inserted under anaesthetic into the right carotid artery of the rats (male, Sprague–Dawley, 250–300 g) to enable serial blood sampling. Animals were then allowed to recover overnight prior to attachment to a swivel and harness system to permit continual, unrestrained blood sampling over the study period. Rats were fasted for 8 h prior to and after dosing, but water was available ad libitum.

2.4.3. Formulation preparation and administration

CIN was dissolved in GMO and OG by addition of a known mass of solid CIN to molten lipid at 37 °C in a 4 mL polypropylene tube to provide 25 mg CIN/g of lipid. The drug was dissolved by repeated vortex mixing, and rotating overnight on a tube roller in a 37 °C incubator. For the OG and GMO formulations, approximately 400 mg of each formulation (∼10 mg CIN) was drawn into a 1 mL syringe, and administered by oral gavage under isoflurane anaesthesia. The dose administered was calculated by accurately weighing the syringe and gavage before and after administration.

The aqueous suspension was prepared by weighing a known mass of drug (∼12.5 mg) into a 1.5 mL Eppendorf tube, and

suspending the solid drug in a solution containing 0.4% Tween 80 and 0.5% (w/v) sodium carboxymethylcellulose in water (0.5 mL) by vortex mixing until uniform. A separate suspension sample was prepared for each rat to enable the absolute dose administered to be accurately determined by assay of the residue in the Eppendorf tube after rinsing the contents of the dosing syringe and gavage back into the Eppendorf after dosing. Approximately 0.4 mL of the suspension was administered by oral gavage under isoflurane anaesthesia.

2.4.4. Sample handling and HPLC analysis for CIN in plasma

Blood samples (0.15 mL) were obtained via the indwelling cannula immediately prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 24 and 30 h for Study 1 and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 57, 72 and 120 h in Study 2. Plasma was separated by centrifugation for 10 min at 2800 × *g*. Cannulas were kept patent by flushing with a small (0.1 mL) volume of 2 IU/mL heparin in saline. The plasma concentration of CIN was assayed by HPLC using a validated extraction procedure, with flunarizine as an internal standard and fluorescence detection, as reported previously (Kossena et al., 2004). The standard pharmacokinetic parameters (mean area under curve (AUC), mean time to maximum plasma concentration (t_{max}), and mean maximum plasma concentration attained (C_{max})) were determined from the individual plasma profiles. The AUC values obtained using the trapezoidal rule and truncated at the final sampling time point, were dose normalised to 40 mg/kg. The relative bioavailability of CIN after administration of the two lipid formulations was calculated in comparison to the aqueous suspension formulation, by assigning a nominal value of 100% to the AUC of CIN after administration of the aqueous suspension. Statistical differences between the pharmacokinetic parameters obtained after the administration of each formulation were tested by one-way analysis of variance with subsequent evaluation of Tukey's least significant differences between groups using a statistical software package (SPSS version 11.5; SPSS, Chicago, IL).

2.5. Histopathology

Histopathological examination of intestinal tissue sections obtained from rats on conclusion of the 120 h OG study (Study 2) was performed to exclude gross histological changes as a mechanism responsible for increased absorption of drug from OG formulations. Immediately prior to sacrifice, under isoflurane anaesthesia, a three centimetre section of the duodenum, jejunum and ileum were excised and stored in formalin buffer for transport to Gribbles Veterinary Pathology (Clayton, Vic., Australia) where a fully blinded histopathological assessment of the intestinal segments was conducted. Microscopic observations of mucus/debris, villus shortening, erosion, epithelial swelling, epithelial flattening and goblet cells, were made using the laboratory's routine procedures, and ranked according to the scheme provided by Swenson et al. (1994).

3. Results

3.1. Phase behaviour of OG and GMO containing cinnarizine

It was stated in Section 1 that GMO and OG form cubic and reverse hexagonal phase, respectively, in excess water at 37 °C. The impact of drug and gastric environment on this phase behaviour was investigated using penetration scans. The photomicrographs in Fig. 2 show that the OG/CIN mixture formed a bright birefringent fan-like texture indicative of hexagonal phase, whilst the GMO/CIN mixture formed a stiff isotropic systems at the interface with water and 0.1N HCl. These results show that the presence of cinnarizine in the lipids did not alter the identity of the phase structure formed by GMO or OG when in contact with excess aqueous solution (water or 0.1N HCl), indicating the cubic or hexagonal phase are likely to form on exposure of these lipids to GI fluids in the subsequent in vivo studies.

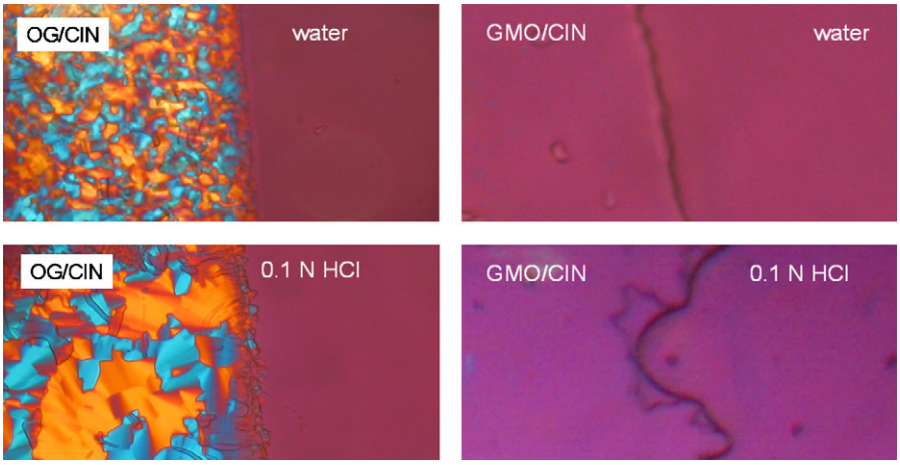

Fig. 2. Photomicrographs of the lipid–water interface viewed through crossed polarising filters. Lipids contained cinnarizine dissolved at 37 °C at 25 mg/g. Left hand images demonstrate the formation of reverse hexagonal phase when the OG/CIN mixture is in contact with excess water or 0.1N HCl as model gastric fluid, while the right hand panels demonstrate the formation of a stiff isotropic cubic phase for the GMO/CIN mixture.

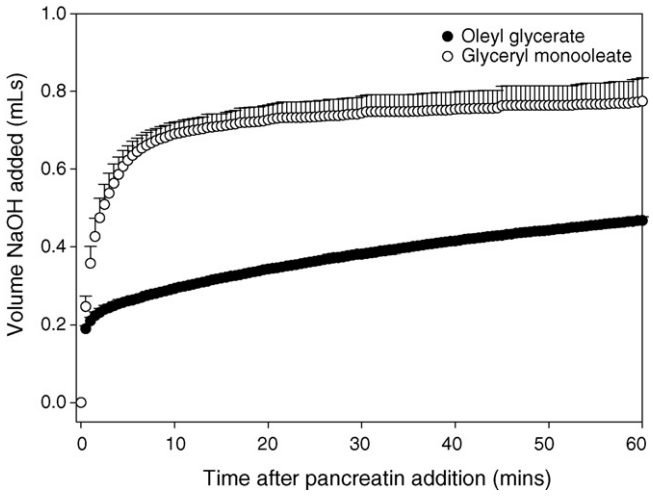

Fig. 3. Titration curves for oleyl glycerate (OG) and glyceryl monooleate (GMO) dispersions on addition of pancreatic lipase, illustrating the rate and extent of lipid digestion (mL of 0.6N NaOH titrated to maintain pH at 7.500 in response to ester hydrolysis). Data are mean ± S.D., $n = 3$. Digestion experiments were conducted at 37.0 ± 0.1 °C.

3.2. In vitro digestion studies

The titration profiles for the OG and GMO dispersions are illustrated in Fig. 3. They both indicate rapid digestion over the initial 5 min, however a significantly lower amount of titrant was consumed for OG than for GMO. In the case of GMO there was virtually no titrant consumed during the following 55 min, while for OG, there was a slow increase in titrant consumption although only approximately 25% more titrant than that consumed over the first 5 min was consumed in the following 55 min period. The titration data were supported by the determination of lipid content over the 60 min digestion period, illustrated in Fig. 4. The profiles indicated that virtually all of the GMO had been digested within the first 5 min period, while for OG, an initial decrease was observed over the first 5 min, followed by a slower, steady decrease in OG concentration in the digestion medium over 60 min.

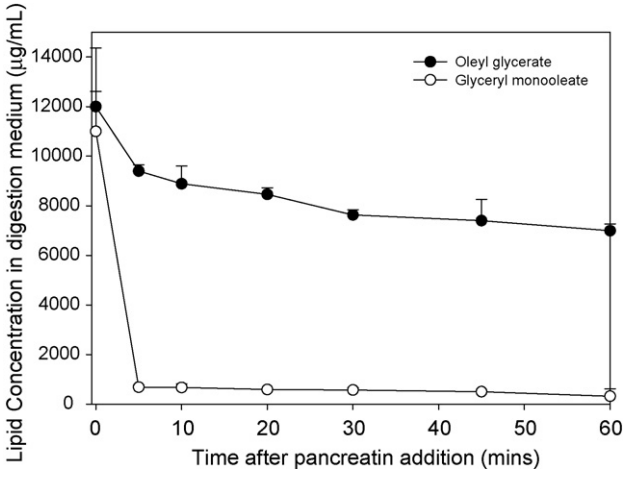

Fig. 4. Time course for lipid digestion determined by concentration of oleyl glycerate (OG) and glyceryl monooleate (GMO) remaining in the digestion medium over time. Data are mean ± S.D., $n = 3$. Concentration of OG determined using HPLC, and concentration of GMO determined using HPTLC.

3.3. In vivo bioavailability

Fig. 5 illustrates the pharmacokinetic profiles for cinnarizine obtained after oral administration as a solution in OG, a solution in GMO and as an aqueous suspension. The pharmacokinetic parameters derived from these profiles, namely C_{max}, T_{max}, AUC to the last time point ($AUC_{0-t\ last}$), and bioavailability relative

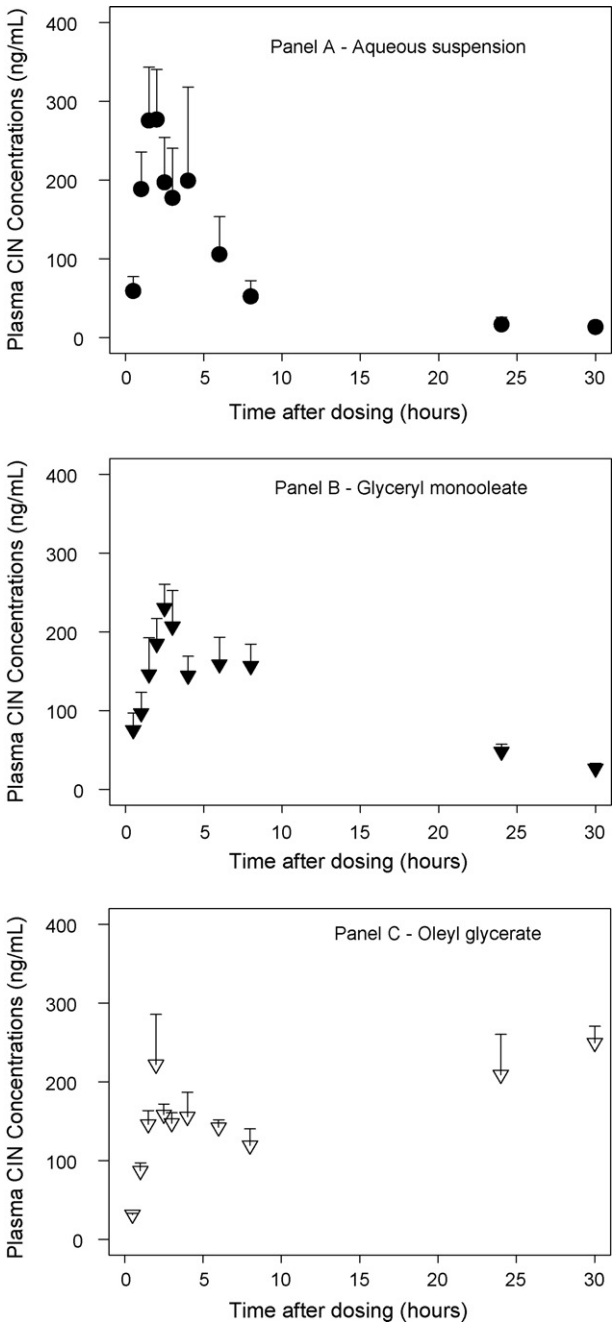

Fig. 5. Study 1: plasma profiles after oral administration of cinnarizine as an aqueous suspension (Panel A) or as solutions in glyceryl monooleate (Panel B) and oleyl glycerate (Panel C) to rats (CIN at 25 mg/g in vehicle, 400 mg of vehicle administered by oral gavage, mean ± S.E.M., $n = 3$).

Table 1
Pharmacokinetic parameters after oral administration of cinnarizine in oleyl glycerate (OG), glyceryl monooleate (GMO) and as an aqueous suspension

Vehicle	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-t\ last}$ (ng/mL h)	F (%)
Study 1 (30 h)					
OG	32.8 ± 2.1	264 ± 49	28.0 ± 3.5	6198 ± 871[a]	210
GMO	33.2 ± 2.3	237 ± 88	3.1 ± 0.6	3449 ± 494[a]	117
Suspension	24.2 ± 1.2	332 ± 73	2.5 ± 1.3	2950 ± 762[a]	100
Study 2 (120 h)					
OG	35.5 ± 1.1	230 ± 47	36.0 ± 0.0	10155 ± 1649[b]	344

Dose, C_{max} and T_{max} are mean ± S.D.; dose normalised AUC data (40 mg/kg) are mean ± S.D., and truncated at the last data point; $n = 3$ for each in Study 1 and $n = 4$ for Study 2.
[a] $t_{last} = 30$ h.
[b] $t_{last} = 120$ h.

to the suspension formulation (F%), are presented in Table 1. All three formulations contained cinnarizine at 25 mg/g, with the actual dose administered ranging from 31.0 to 35.7 mg/kg for the lipid formulations, and between 23.4 and 25.7 mg/kg for the suspension.

The aqueous suspension provided for reasonable CIN exposure over the first 8 h (T_{max} at 2.5 h) consistent with previous studies in beagle dogs (Tokumura et al., 1987). Over the same time scale the plasma profiles of CIN after administration in the GMO and OG formulations were similar to the suspension, with a peak in plasma concentration at approximately 2–3 h. For the GMO formulation, however, the subsequent decrease in plasma concentration was delayed compared to the suspension, although the concentration of CIN in plasma had declined to relatively low levels (26.6 ± 6.5 ng/mL) at 30 h post-administration. In contrast, the plasma concentrations of CIN after administration of the OG formulation at 24 and 30 h were similar to, and for some subjects greater than those at 2 h, and appeared to be increasing with time at 30 h, suggesting that a second absorption event was occurring. This resulted in an overall 2.1-fold increase in AUC up to 30 h for CIN when administered in the OG formulation, relative to that of the suspension formulation.

In order to (i) verify that a second absorption event was occurring at 30 h after administration, (ii) determine whether the duration of drug absorption continued beyond 30 h, and (iii) better understand the plasma profiles between 8 and 24 h time points after administration of the OG formulation, an equivalent oral bioavailability study was performed (Study 2), in which the intermediate plasma samples were acquired and further samples taken up to 120 h after dosing.

The plasma profile obtained for CIN in Study 2, after dosing the same OG formulation as in Study 1, is illustrated in Fig. 6. The initial peak in CIN concentration was again evident at approximately 2 h after dosing. The plasma concentrations then declined over the following 16 h before rising again at approximately 18 h. In agreement with the plasma profile in Study 1, the plasma concentration at 30 h was approximately equivalent to the plasma concentration at 2 h. The second peak in the plasma profile described an increase in drug concentration in plasma to a maximum at the 36 h time point. The plasma concentration subsequently decreased back to low levels over the following 72 h period.

The additional data points taken between 8 and 24 h in Study 2 revealed that the second rise in plasma profile did not commence until 18 h after dosing, or 10 h after completion of the fasting period, indicating that this is unlikely to be associated with the prandial state of the subjects. Likewise, there was no correlation between day/night or light/dark cycles and the time of the second peak.

Using the data obtained from the complete plasma profiles for CIN after administration of the OG formulation obtained from Study 2, and the data from the GMO and suspension from Study 1, better comparisons between the formulation effects can be made. The T_{max} value for the GMO and suspension formulations were essentially the same at approximately 3 h, while that for OG formulation in the 120 h study was determined to be 36 h (all animals displayed maximum plasma concentration at this time point). The extended duration of absorption compared to the suspension and GMO formulations provided a much greater value for the truncated $AUC_{0-t\ last}$ of 10155 ± 1649 ng/mL h, and consequently, relative bioavailability was nearly 3.5 times greater than that of the suspension formulation ($p = 0.018$) and 3 times greater than the GMO formulation ($p = 0.008$). The GMO formulation on the other hand did not provide a statistically significant difference in bioavailability compared to the suspension ($p = 0.62$).

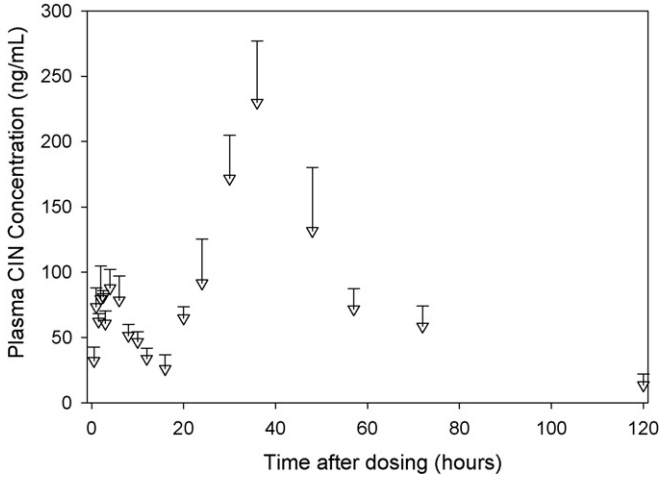

Fig. 6. Study 2: plasma profiles after oral administration of cinnarizine as solution in oleyl glycerate to rats (CIN at 25 mg/g in vehicle, 400 mg of vehicle administered by oral gavage, mean ± S.E.M., $n = 4$).

Table 2
Randomised, blinded histopathological results on excised segments of rat intestine after administration of the OG formulation, equivalent to an approximate dose of 1.5 g OG/kg of rat body weight

	Received oral dose of OG			No exposure	
	Rat A	Rat B	Rat C	Rat E	Rat F
Duodenum					
Mucus/debris	0	1	1	1	0
Villus shortening	0	1	2	1	1
Erosion	0	1	2	1	0
Epithelial swelling	1	0	2	0	0
Epithelial flattening	0	0	0	0	0
Goblet cell	0	0	0	0	0
Jejunum					
Mucus/debris	0	2	1	2	1
Villus shortening	1	1	0	2	2
Erosion	0	1	1	1	2
Epithelial swelling	0	1	1	1	1
Epithelial flattening	1	1	0	2	2
Goblet cell	1	0	1	2	2
Ileum					
Mucus/debris	1	3	1	0	3
Villus shortening	1	3	2	2	3
Erosion	0	3	1	1	3
Epithelial swelling	0	2	0	0	1
Epithelial flattening	0	0	1	2	3
Goblet cell	1	1	1	2	1

Rat intestinal tissue samples ranked 0–3 for each criteria in blinded fashion (0 = no effect, 3 = severe effect), according to Swenson et al. (1994).

3.4. Clinical observations and histopathology after oral administration of OG

Despite the relatively large mass of formulation dosed to the rats in the case of both OG and GMO (350–400 mg), no overt signs of discomfort were observed in any animals. All animals were observed to eat and drink in a normal fashion, and no signs of diarrhoea or constipation were detected for any animals over the study duration.

The histopathology results obtained for different sections of intestine excised from the rats at sacrifice in Study 2 for treated (120 h after dosing the OG formulation) and non-treated rats are provided in Table 2. There was no observable difference between the treatment and control groups based on the ranking data, indicating that no observable damage or changes in pathology of the duodenum, jejunum or ileum had occurred after exposure to 1.5 g/kg oleyl glycerate. One rat had 'severe' ratings for several criteria in the ileum sample; however this was also evident in one control rat. We conclude from this data that absorption of CIN in the pharmacokinetic study was not mediated by irreversible pathological changes in the intestine due to the presence of oleyl glycerate.

4. Discussion

The ability of lipids to improve the oral bioavailability of poorly water soluble drugs *via* improved drug solubilization in the GIT is well recognised (Humberstone and Charman, 1997). Formulation lipids and lipid digestion products interact with endogenous surfactants such as bile salts and phospholipids in the GIT to form a range of colloidal and liquid crystalline structures that help to reduce precipitation and maintain drug in a solubilized state, thereby facilitating the mass transport of poorly water soluble drug through the aqueous GIT lumen to the intestinal surface.

Recently there has been growing interest in the potential pharmaceutical utility of polar lipids that form liquid crystalline structures in excess water (Drummond and Fong, 1999; Shah et al., 2001). Of particular interest are the viscous bicontinuous cubic and reverse hexagonal phases, whose structures provide a diffusional barrier to drug release when the drug is incorporated into the matrix on formation, hence these systems have potential as sustained release matrices. However, the influence of liquid crystalline structure on drug absorption after oral administration of liquid crystal-forming lipids, and whether controlled release effects may be obtained in vivo has not been described. Kossena et al. have shown that the absorption of cinnarizine, administered intraduodenally in a cubic phase liquid crystalline matrix, was sustained for a longer duration than from a suspension formulation (Kossena et al., 2004) in bile duct ligated rats to minimize the influence of lipid digestion on liquid crystal integrity, and hence it is of interest to determine whether similar behaviour can be achieved after oral administration. Liquid crystalline structures can be formed by dietary lipids such as GMO, however the rapid digestion of such lipids, and consequent degradation of the liquid crystalline matrix, has arguably been the major limitation to the study of the impact of liquid crystal formation on oral drug absorption (Shah et al., 2001).

The digestibility of glyceride-based lipids with ester functional groups is likely to be a general limitation to maintaining

liquid crystalline structure after oral administration. As an alternative, a number of other classes of lipids are able to form viscous liquid crystalline structures in excess water, and may be less susceptible to digestive processes. We recently reported the ability of alkyl glycerates to form reverse hexagonal phase in excess water, and characterized the controlled release of hydrophilic and hydrophobic drugs from the liquid crystalline matrix of oleyl glycerate (OG) (Boyd et al., 2006). Oleyl glycerate is structurally very similar to GMO (Fig. 1), but was synthesised with a glycerate rather than glyceryl ester group. This point of differentiation was the impetus for our study into its influence on the oral bioavailability and pharmacokinetics of poorly water soluble drugs.

The plasma profiles obtained after administration of CIN in OG provided significant and very unpredictably prolonged exposure to CIN. A double peak phenomenon was also observed. The initial peak in plasma CIN concentration is similar in duration to those observed for both the suspension and GMO formulations, and is likely to reflect initial exposure to CIN released from the surface of the formulation and prior to establishment of the liquid crystalline structure. However, a second peak in the plasma profile for the OG formulation was evident after 18 h, and provided a sustained plasma concentration for CIN over the subsequent 48 h. Both the presence and form of this second peak in the plasma profile require discussion. First, it should be noted that no second peak was evident in the plasma profiles for the GMO formulation. In addition, a previous bioavailability study on CIN, administered as a suspension and as a solution in oleic acid to beagle dogs (Tokumura et al., 1987), did not show the second peak phenomenon. Likewise, CIN administered to rats as an intravenous formulation as a cyclodextrin complex (Krise et al., 1999) did not show a second peak in the plasma profiles. Taken together, these findings indicate that the second peak in the plasma profile is unlikely to be due to a distribution or reabsorption phenomena, indicating that it is most likely a second absorption phase of drug from the gastrointestinal tract.

The elimination half life of CIN is less than 3 h in rats (Krise et al., 1999), dogs (Tokumura et al., 1987) and humans (Paton and Webster, 1985), therefore in order for the second peak in the plasma profile for the OG formulation to be an absorption event, and in consideration of the very long elevated plasma concentrations observed in Fig. 6 the drug must reside in the gastrointestinal tract for extended periods of time up to 72 h. The apparent retention of the OG formulation in the GIT compared to the GMO formulation is consistent with the digestion studies, which showed that GMO is significantly more susceptible to digestive processes than OG (Figs. 3 and 4). GMO cubic phase has been shown to be mucoadhesive (Nielsen and Hansen, 1998) and the structural similarity between GMO and OG, in terms of the same number of hydroxyl groups available for hydrogen bonding to mucus membranes, would suggest that OG would also exhibit mucoadhesion, although this is yet to be shown.

The timing of the second peak was remarkably consistent across the seven animals that received the OG formulation. The second rise in plasma concentration did not correlate with either time of access to food, or the light/dark cycles suggesting that the timing of the second peak is determined by the nature of the formulation and physiological processes acting upon it. The release of hydrophobic drugs from OG is diffusion controlled (Boyd et al., 2006), but will also be limited by saturation of the surrounding release environment. Therefore very slow release of drug from the formulation would be expected when the formulation is intact. However, it is known that changes in liquid crystalline phase structure, and hence the dimensions of the internal aqueous domains, impact on the diffusivity of molecules through the matrix altering the drug release rate in liquid crystalline systems (Chang and Bodmeier, 1997; Drummond and Fong, 1999; Farkas et al., 2000; Boyd et al., 2006). The release rate of hydrophobic drugs from OG hexagonal phase compared to GMO cubic phase was several fold slower, so whilst the structure of the matrix will be a contributing factor to the rate of drug release from the matrix and hence appearance in plasma, this difference would not lead to the dramatic changes in rate of drug appearance in plasma observed for the second peak in the plasma profiles. It has been shown previously that the reverse hexagonal phase formed by OG in excess water tolerates up to 25% oleyl glycerate before converting to the low viscosity reverse micellar phase (Boyd et al., 2006). Thus, the digestion of OG to form oleyl alcohol will eventually lead to a change to the low viscosity reverse micellar phase, a matrix less able to sustain drug release, which may also be a contributing factor to the temporal aspects of the second peak in the plasma profile after administration of the OG formulation.

In light of the results obtained in this study and the discussion above, we propose that in order to observe the extended plasma profiles observed with OG as a formulation lipid there are three necessary factors. First, the formulation must form a sustained release matrix with the ability to substantially delay drug release into the GIT environment unlike, for example, a simple emulsion where rapid drug release via partitioning can occur. Second, the formulation must resist digestive processes to provide a persistent matrix from which drug may be slowly released, and third, there must be a means of retaining the formulation in the GIT, such as mucoadhesion in order to prevent bulk passage through the GIT over a relatively short time period. GMO exhibits the first and third feature, but its rapid digestion indicates that it is not able to provide a persistent matrix. Whilst the absolute role of each of these factors is not clear in the current study, each is likely to be a contributing factor and further work is now required to better understand and optimize the interplay between these factors, through alteration of lipid structure, liquid crystalline phase behaviour and dose, and the physicochemical properties of the drug, to progress the use of these systems as potential sustained oral delivery systems for poorly water soluble drugs.

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