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Research Paper

Phytantriol and glyceryl monooleate cubic liquid crystalline phases as sustained-release oral drug delivery systems for poorly water-soluble drugs II. In-vivo evaluation

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Abstract

Objectives Lipid-based liquid crystals formed from phytantriol (PHY) and glyceryl monooleate (GMO) retain their cubic-phase structure on dilution in physiologically relevant simulated gastrointestinal media, suggesting their potential application as sustained-release drug-delivery systems for poorly water-soluble drugs. In this study the potential of PHY and GMO to serve as sustained-release lipid vehicles for a model poorly-water-soluble drug, cinnarizine, was assessed and compared to that of an aqueous suspension formulation.

Methods Small-angle X-ray scattering was used to confirm the nanostructure of the liquid-crystalline matrix in the presence of the selected model drug, cinnarizine. Oral bioavailability studies were conducted in rats, and disposition of lipid and drug in segments of the gastrointestinal tract was determined over time. Differences in the digestibility and stability of formulations under digestion conditions were investigated using an in-vitro lipolysis model.

Key findings The oral bioavailability of cinnarizine using the PHY formulation was 41%, compared to 19% for the GMO formulation and 6% for an aqueous suspension. The PHY formulation provided a T_{max} for cinnarizine of 33 h, with absorption apparent up to 55 h after administration. In contrast, the T_{max} for the GMO formulation was only 5 h. The PHY formulation was retained in the stomach for extended periods of time, with 56% of lipid remaining in the stomach after 24 h, in contrast to less than 1% of the GMO formulation after 8 h, suggesting that gastric retention was a key aspect of the prolonged period of absorption, which correlated with the formulations' relative susceptibility to in-vitro lipolysis and degradation.

Conclusions PHY provides a dramatic sustained-release effect for cinnarizine on oral administration, which is linked to gastric retention of the formulation and its ability to resist digestive processing. Poorly digested liquid crystal lipid formulations therefore offer a novel class of sustained-release matrices for oral administration.

Keywords cubic phase; in vivo; lipid formulation; lipophilic drug; liquid crystal; sustained release

Introduction

Lipid-based vehicles are an increasingly utilised approach in cases where reduced aqueous solubility and dissolution rate limit drug bioavailability. Their primary function is to maintain the drug in a solubilised state prior to and during digestion of the lipids. Lipid digestion products combine with endogenous bile components to generate a high solubilisation capacity environment for drugs in the gastrointestinal fluid and therefore a reservoir from which drug absorption can occur. Systems containing triglycerides and/or partially digested triglycerides as the lipid components (along with surfactants and co-solvents) are commonly employed, and characterisation of their in-vitro and in-vivo behaviour has received the most attention in the literature.[1,2] However, aside from the potential for delaying gastric emptying, these formulations are not recognised as having

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an inherent capability for slow release of drugs from the formulation, and hence their potential to modulate the temporal aspects of drug absorption is limited.

Self-assembled liquid crystalline (LC) structures, such as the bicontinuous cubic (Q_2) and inverse hexagonal phases (H_2), which are formed by various amphiphilic lipids in excess water at high dilution, have been identified as potential sustained-release drug-delivery vehicles.[3] Incorporation of drugs into the complex internal domains of the Q_2 structure can facilitate diffusion-controlled release of drugs into the surrounding external aqueous environment.[4,5] In an accompanying paper,[6] we have demonstrated that glyceryl monooleate (GMO) and phytantriol (PHY) maintain their cubic phase structure in the presence of model gastrointestinal fluids. This behaviour suggests their potential to provide slow release of drugs in the gastrointestinal tract (GIT).

Despite their potential for sustained release, there have been very few in-vivo studies of these materials. Boyd *et al*. have shown that GMO cubic phase formulations have only a limited ability to sustain the absorption of the poorly water-soluble drug cinnarizine (CZ) after oral administration.[7] Digestion of the lipid vehicle was suggested to be responsible for the poor sustained-release capability, supported by the fact that the less-readily digested GMO analogue oleyl glycerate was able to sustain the absorption of CZ under similar conditions. Hence it appears that digestibility is one critical hurdle that needs to be overcome to enable progress of LC systems towards oral sustained-release vehicles.

Phytantriol is a non-digestible amphiphilic lipid that also forms a viscous Q_2 phase in excess water[8] but has not been reported as an oral sustained-release matrix for poorly water-soluble drugs. In the current study, oral bioavailability studies were undertaken using the model poorly water-soluble drug CZ using PHY and GMO cubic-phase-forming lipid vehicles. To ensure that CZ would not influence the phase behaviour of the LC *in vivo*, the nanostructure of the LCs was first confirmed using small-angle X-ray scattering (SAXS). CZ was administered orally as lipid solutions in PHY and GMO, on the expectation, based on the results in the accompanying paper,[6] that cubic phase would form in GIT fluids. Plasma concentrations were measured over time to elucidate whether the LCs were able to facilitate sustained drug absorption and the location of the LC formulation components was determined over time to correlate disposition of the formulation with pharmacokinetic profiles. Lastly, the potential digestibility of PHY in comparison to GMO was confirmed in a lipolysis model.

Materials and Methods

Materials

Phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) was sourced from BASF (Washington, NJ, USA). The water content of PHY was less than 0.034% (using Karl Fischer titration). Glyceryl monooleate (Myverol 18–99) was kindly donated by Kerry Scientific (Norwich, USA), and had a GMO content of 60.9% w/w (monoolein water content of $\leq$1%[9]). Both lipids were used as obtained without further processing or purification. Model bile salt solutions were prepared using either egg yolk lecithin (consisting of approximately 60% phosphatidyl choline by dry weight) or lysophosphatidyl choline (Sigma Co, St Louis, MO, USA). Sodium taurodeoxycholate (NaTDC), Trizma maleate, CZ, flunarizine, porcine pancreatin (8 $\times$ USP specification), sodium azide, sodium carboxymethylcellulose, benzyl alcohol, Tween 80 and trichloroacetic acid (TCA) were obtained from Sigma Co. Ammonium dihydrogen orthophosphate was from BDH (Poole, UK). All other chemicals were of AR quality. Acetonitrile (ACN), dimethyl sulfoxide (DMSO) (Ajax Chemicals, NSW, Australia), tert-butyl methyl ether (TBME), ether, chloroform and hexane (95% v/v) (Mallinckrodt, NJ, USA) were of HPLC grade. Ethanol (99%) purity was obtained from CSR distilleries (Yarraville, Victoria, Australia). Water was obtained from a Milli-Q filtration/purification system (Millipore, MA, USA).

Bioavailability of cinnarizine in rats

Preparation of cinnarizine formulations

The intravenous (IV) formulation consisted of 0.55 mg/ml CZ dissolved in a co-solvent system comprising 10.6% v/v DMSO, 0.4% v/v Tween 80 and 89% v/v phosphate buffer pH 4 (36 mM disodium hydrogen orthophosphate and 22 mM potassium dihydrogen orthophosphate). To minimise degradation of CZ in the formulation at low pH,[10] the IV formulation was used within 24 h of preparation. Oral formulations consisted of CZ dissolved in molten PHY and GMO at 37°C equivalent to 7 mg/g and an aqueous suspension of CZ (7 mg/g) as a control. The aqueous suspension comprised 0.5% w/v sodium carboxymethylcellulose, 0.5% v/v benzyl alcohol and 0.4% v/v Tween 80 in saline.

Confirmation of phytantriol and glyceryl monooleate phase behaviour in the presence of cinnarizine using small angle X-ray scattering

To ensure that the Q_2 nanostructure would be maintained in the presence of CZ, the nanostructure of dispersed PHY and GMO was assessed using SAXS. To facilitate rapid equilibration with the surrounding media, the LC phases were prepared as dispersed emulsion-like systems (the likely state of a lipid formulation under intense shearing in the stomach soon after administration), as described in the accompanying paper.[6] The PHY and GMO dispersions comprised 10% w/v of lipid $\pm$ CZ. The particle size of the dispersed LC systems was measured using a Malvern Instruments Nano-ZS Zetasizer (Malvern, UK) at 37°C, revealing a particle diameter of 240 $\pm$ 3.2 and 190 $\pm$ 2.9 nm for dispersed PHY and GMO LCs, respectively, with a polydispersity index of <0.4. SAXS measurements were then undertaken on a Bruker Nanostar system as described in the accompanying paper.[6]

Rat surgical procedure

Male Sprague Dawley rats weighing between 280 and 380 g were used for the IV and oral dosing experiments. Studies were approved and conducted in accordance with the guidelines of the Victorian College of Pharmacy Animal Ethics Committee. Rats were anaesthetised via inhalation of isoflurane (2.5% v/v; Abbott Laboratories, NSW, Australia) for the duration of the surgical procedure and cannulas

inserted into the jugular vein and carotid artery. Polyethylene tubing of 0.96 × 0.58 mm (o.d. × i.d.) was advanced approximately 2.5 cm from the site of cannulation into the jugular vein to enable IV administration of the formulation. The cannula was secured to the vein and the process repeated for the carotid artery to allow for the unhindered collection of blood after IV and oral administration. Rats were connected to a harness and swivel system and allowed to recover overnight prior to dosing. Rats were also fasted for at least 12 h prior to and 8 h after dosing. However, water was provided ad libitum.

Administration of formulations and sample collection

For the IV studies, 1 ml of formulation was administered via the jugular vein cannula over a 2 min infusion period and the cannula subsequently flushed with 2 IU/ml sodium heparin saline solution to ensure no residual CZ remained. Immediately after infusion a 200 μl blood sample was taken at $t = 0$ with further samples obtained 5, 10, 20, 30, 45, 60, 240, 360, 480 and 1440 min after dosing. For the oral administration studies 300 mg of each of the oral lipidic formulations (2.1 mg CZ) were dosed via an oral gavage. The dose was accurately assessed by measuring the difference in the mass of lipid in the gavage and syringe before and after dosing. After oral administration of the suspension, the gavage and syringe were rinsed with 2 : 1 v/v ACN : ethanol. The ACN/ethanol rinse was subsequently assayed, via HPLC, for drug content to evaluate the dosed quantity of suspension. Blood samples (250 μl) were obtained up to 100 h after oral administration. In all cases cannulas were flushed with 2 IU/ml sodium heparin saline solution after each sample to ensure patency. Collected blood samples were transferred to 1.5 ml Eppendorf tubes containing 10 IU sodium heparin and centrifuged for 5 min at 6700*g*. A quantity of 100 μl of plasma was collected and stored at −20°C until assayed for CZ content.

Sample processing and HPLC analysis of cinnarizine in plasma

CZ plasma samples from both IV and oral studies were processed using a validated plasma extraction assay, and flunarizine was used as the internal standard, in a similar manner to that previously outlined in the literature.[11,12] The HPLC system comprised a C_{18} 5 μm, 150 × 3.9 mm Symmetry RP column (Waters, Milford, MA, USA) and a 7 μm, 15 × 3 mm Brownlee RP guard column (Alltech Associates, Deerfield, IL, USA). The column was coupled to an autosampler (Model 717 plus), a Model 610 fluid unit, Model 600 fluid controller (Waters) and a RF-10A XL fluorescence detector supplied by Shimadzu (Shimadzu Corp., Kyoto, Japan). The detector was set at excitation and emission wavelengths of 249 and 311 nm, respectively. Data were recorded and integrated using Empower 2 personal chromatography data software (Waters). The mobile phase, comprising 48 : 52 v/v of ACN and 20 mM ammonium dihydrogen orthophosphate buffer, was delivered at 1 ml/min at room temperature. An injection volume of 100 μl was used with the CZ peak eluting at approximately 6.7 min and flunarizine at 7.7 min. The results were plotted as CZ concentration (ng/ml) (semi-log plot for IV data) versus time (h) with errors calculated as standard error of the mean (SEM). After oral administration, C_{max} and T_{max} were determined from the normalised data and the truncated area under the curve ($AUC_{0\text{-}t\text{-}last}$) was calculated using the trapezoidal rule. After IV administration the elimination rate constant (k), clearance (CL), volume of distribution (Vd_{β}) and area under the curve ($AUC_{0\text{-}\infty}$) were also calculated using standard pharmacokinetic methods.[13] Oral bioavailability (BA) was calculated by comparison of the dose normalised IV and oral AUCs, and expressed as percentage absolute BA (F%). SPSS for Windows (Version 15.0, SPSS, Chicago, IL, USA) was used to statistically analyse differences in the data using a one-way analysis of variance with Tukey's multiple comparison, with statistical significance assumed when $P \leq 0.05$.

Assessment of location of formulation components in the gastrointestinal tract

To assess the location of components of the PHY and GMO formulations (lipid and drug) in the GIT over time, sections of the GIT were removed and assayed for lipid and drug content. Prior to administration, rats were fasted for at least 12 h and given free access to water to allow gastrointestinal transit of previously ingested material. A quantity of 300 mg of the PHY and GMO formulations (containing 7 mg/g CZ, as used in the oral BA studies) was dosed orally via gavage ($n = 12$ rats per formulation). Rats remained fasted throughout the study but were given free access to water. At 1 h post dose, three rats from each dose group were sacrificed (sodium pentobarbital via intra-cardiac puncture) and the stomach, duodenum (10 cm), jejunum (30 cm) and ileum (20 cm) were removed along with the remaining intestine and faeces. This process was repeated at 4 h (stomach, intestine and faeces), 8 h (stomach only) and 24 h (stomach, intestine and faeces) post-dose.

Each section was opened and the internal lining was scraped clean with a scalpel blade to collect the contents. The contents were mixed in 2 : 1 v/v ACN : ethanol to dissolve both lipid and drug. Faecal pellets were also prepared in a similar manner. The mixtures were vortexed and centrifuged for 15 min at 3500*g* and the supernatant subsequently collected and stored at −20°C until analysed. Samples were assayed for lipid content using a validated high performance thin layer chromatography (HPTLC) densiometric method as described by Sek *et al*.,[14] using the modified development steps outlined in Table 1. Developed plates were subsequently stained using a solution containing 10 : 8 : 82% w/v/v cupric

Table 1 Solvent mixtures and development steps undertaken in the separation and quantitation of phytantriol and glyceryl monooleate derived from the contents of gastrointestinal segments obtained after oral dosing of the lipid formulation to rats

Step	Percentage ether	Percentage chloroform	Percentage hexane	Percentage methanol/ water 97 : 3	Migration distance (mm)
1	0	0	100	0	55
2	0	73	0	27	25
3	0	85	0	15	50
4	80	0	20	0	45
5	25	0	75	0	55

sulfate (pentahydrate):85% phosphoric acid : water and quantified densitometrically using a deuterium UV light source set at a wavelength of 370 nm. Data are presented as intensity versus migration distance, where PHY and GMO migrated 32 and 36 mm, respectively, from the end of the plate. CZ content in each section of the GIT was quantified using the HPLC system previously described, with a slight difference in mobile phase (50 : 50 v/v ACN:20 mM ammonium dihydrogen orthophosphate).

Absorption of phytantriol from the gastrointestinal tract

To address a lack of quantitative recovery in the studies conducted to determine the location of PHY in the GIT over time, an effort was made to determine whether PHY was absorbed from the GIT by analysis of plasma samples for PHY concentration after oral administration. In order to obtain a PHY concentration in plasma sufficiently high to enable detection by HPTLC, a concomitantly high quantity of PHY was expected to be required in the small intestine (making the assumption that absorption of phytantriol would occur in the small intestine, as is typically the case for other lipophilic substances). A complication of oral administration of relatively large quantities of lipids, however, is the likelihood of delayed gastric emptying and therefore slow delivery of material to the absorptive site. In an attempt to circumvent these problems, PHY was solubilised into a low-viscosity micellar solution, on the assumption that more rapid gastric emptying and more effective absorption would occur, enabling an opportunity to detect PHY in plasma if absorption occurred. The formulation was prepared by dispersing 90 mg of PHY in 3 ml of mixed micellar solution containing 5 mM phosphatidyl choline and 20 mM sodium taurodeoxycholate in buffer. The formulation was administered by oral gavage to rats, and blood samples (1 ml) were collected 1, 4 and 8 h after administration via a cannula inserted into the carotid artery. Blood samples were centrifuged for 10 min at 6700*g* after which 500 μl of plasma was collected and extracted using 10 ml of TBME. The solvent was evaporated and sample reconstituted in 2 : 1 v/v ACN : ethanol prior to being assayed for PHY using the validated HPTLC method as described in the previous section.

In-vitro digestion studies

In-vitro digestion studies were undertaken using a digestion model based on a pH-stat autotitrator (Radiometer, Copenhagen, Denmark) as described previously,[15–17] using digestion buffer and simulated fasted intestinal fluid. To facilitate efficient mixing and enzyme/substrate binding, the viscous PHY and GMO LCs were prepared as dispersions, with 1 g of lipid dispersed in 9 ml of simulated intestinal fluid (containing 1% w/v Pluronic F127 as a stabilising agent) and ultrasonicated (Misonix XL 2000, Misonix, NY; 2 s pulse, 2 s pause for 30 min). On addition of pancreatin (20 000 TBU), the pH-stat titrated the digestion mixture with 0.2 M NaOH, in order to maintain the system at pH 7.500 $\pm$ 0.002. Digestion was allowed to proceed for 60 min, after which the degree of enzymatic digestion of the lipid was calculated from the volume of NaOH used to neutralise the fatty acids (expressed in mmol) liberated during the digestion process. To account for the fatty acids released from the digestion of phosphatidyl choline by phospholipase A_2 (also found in the pancreatin mixture), blank digestions in the absence of lipid were also performed and subtracted from the total titrant consumed during the digestion of the formulation.

PHY does not possess fatty acid moieties that can be detected using the pH-stat titration system. Consequently, pre- and post-digestion samples were analysed via HPTLC, with changes to the intensity or migration distance of the PHY bands appearing on the HPTLC plate being used to indicate whether the lipid was being chemically altered through the digestion process. Immediately before and after subjecting the PHY LC dispersions to the in-vitro digestion protocol, 1 ml of the digestion mixture was transferred to an Eppendorf tube containing 10 μl of 0.5 M 4-bromophenylboronic acid in methanol as a lipase inhibitor[16] and analysed using a validated HPTLC method as previously described.

Results

Effect of increasing cinnarizine concentration on phase nanostructure

To confirm retention of the Q_2 nanostructure in the presence of CZ, the phase behaviour of Q_2 phases of PHY and GMO in excess aqueous phase was assessed by SAXS. CZ had no effect on the space group of PHY and GMO Q_2 phases (*Pn3m* and *Im3m*, respectively) as CZ concentrations were increased up to saturation levels in the lipid (7 and 24 mg/g, respectively). This was evidenced by close-to-identical scattering profiles with increasing CZ concentration for PHY (Figure 1a) and GMO (Figure 1b). However, when GMO was saturated with CZ, the appearance of a shoulder peak between the second ($\sqrt{4}$) and third ($\sqrt{6}$) Bragg peaks was observed, suggesting the emergence of another phase (most likely the first peak for a H_2 phase). The significance of this to the current study is not high because the bioavailability study was conducted with CZ at 7 mg/g in both lipids (~25% of saturation in GMO), at which concentration the H_2 peak is not apparent, indicating 'pure' cubic phase. Presumably the higher concentration of drug is sufficient to disrupt the packing of the chains within the cubic phase bilayer as the concentration approaches saturation. The potential for phase change on addition of drug is an important issue for those materials that needs to be assessed on a case-by-case basis. Nevertheless, the lattice parameters for PHY and GMO Q_2 phases did not change significantly as the concentration of CZ was increased to saturation in each lipid (Figure 1c). The SAXS findings correlate well with crossed polarised light microscopy observations in the accompanying paper,[6] which showed a Q_2 LC in all PHY and GMO systems in the presence of CZ. These results suggest that the presence of CZ will not result in a significant change in phase structure on oral administration.

Pharmacokinetics of cinnarizine after oral and intravenous administration of test formulations

Intravenous pharmacokinetics

For calculation of absolute bioavailability after oral administration of lipid-based formulations and the suspension, the IV pharmacokinetics of CZ were first studied. The calculated PK parameters were comparable to those in previous studies

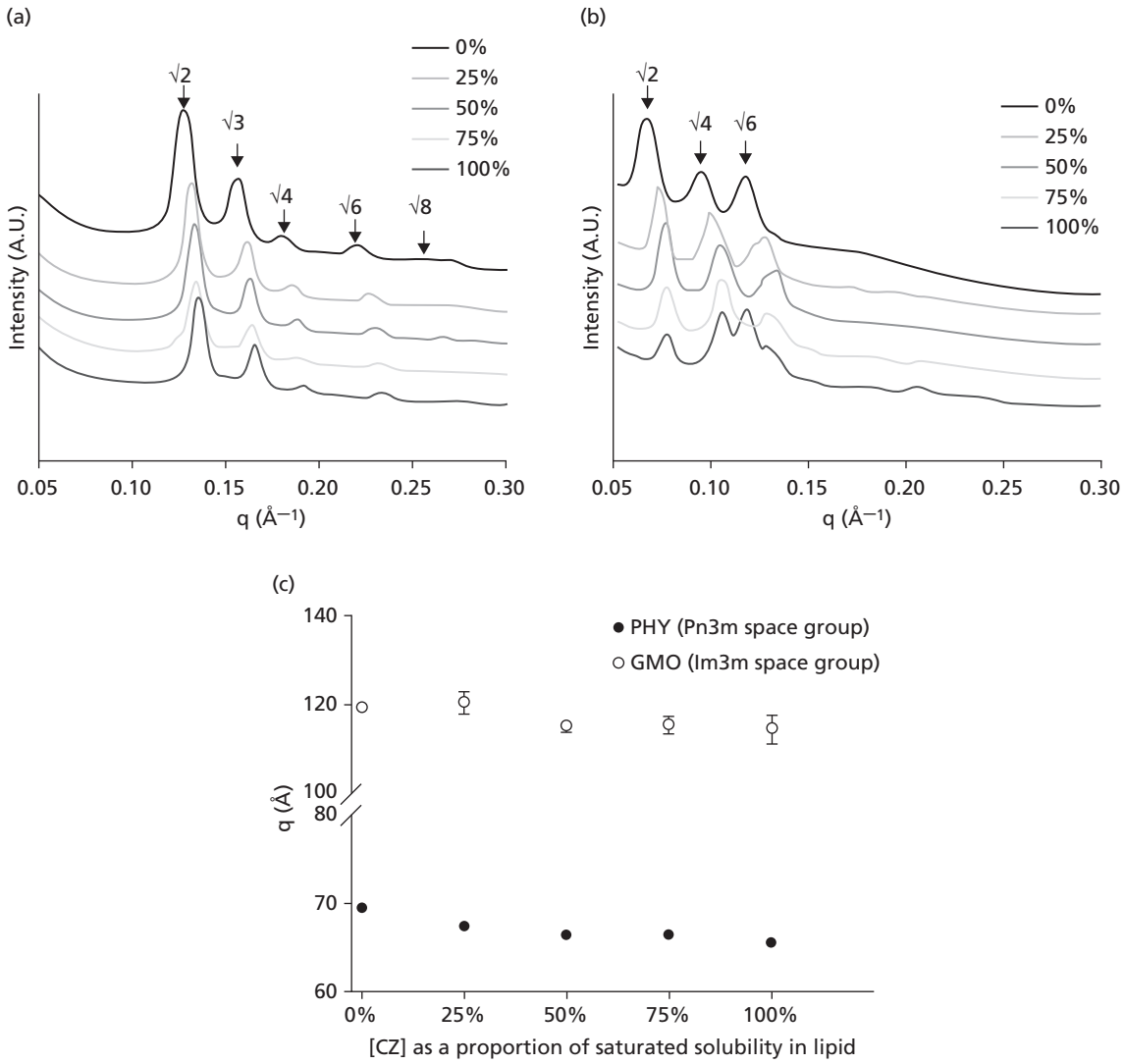

Figure 1 Small-angle X-ray scattering profiles for phytantriol and glyceryl monooleate. The profiles are shown for (a) phytantriol (PHY) and (b) glyceryl monooleate (GMO) dispersions in the presence of increasing concentrations of cinnarizine (CZ; %) up to saturated solubility. The saturated solubility of CZ in PHY and GMO was 7 and 24 mg/g, respectively. The minimal variability in lattice parameter with increasing CZ concentration in both lipid systems (c) indicates that CZ does not significantly affect the integrity of the Q_2 phase.

by Krise *et al*. on IV administration of CZ in rats.[11] The calculated CL (24.3 ± 4.0 ml/min per kg) was similar to that calculated from the reported study (19.0 ml/min per kg) (see Table 2). However, the relatively longer sampling period in the current study (24 h) resulted in a longer terminal half-life and therefore larger Vd_β than that reported in the previous study, where samples were only obtained for 4 h after administration (Vd_β = 3.6 l/kg, $t_{1/2}$ = 2.2 h).

Table 2 Pharmacokinetic parameters obtained after the intravenous administration of cinnarizine

Pharmacokinetic parameter	This study	Literature[a]
AUC (0–∞) (ng/ml.h)	1364 ± 205	–
Terminal half-life ($t_{1/2}$) (h)	7.3 ± 2.2	2.2
Clearance (CL) (ml/min per kg)	24.3 ± 4.0	19.0
Volume of distribution (Vd_β) (l/kg)	14.2 ± 3.4	3.6

Cinnarizine concentration in formulation at 0.55 mg/ml, 1 g vehicle (n = 4, mean ± SEM). [a]Source: Krise *et al*.[11]

Oral pharmacokinetics and bioavailability of cinnarizine formulations

Mean dose-normalised CZ plasma profiles obtained after the oral administration of CZ in 300 mg of each vehicle are shown in Figure 2, with the derived pharmacokinetic parameters from these profiles in Table 3. Although plasma samples were taken for 48 h for the suspension and GMO formulation, and 103 h for the PHY formulation, plasma concentrations in all cases fell below the limit of quantitation (4 ng/ml) before the completion of the sampling regime. The plasma profile after administration of the CZ suspension exhibited a mean T_{max} of only 1.0 h and C_{max} of 97 ± 13 ng/ml. Administration of the GMO formulation resulted in a

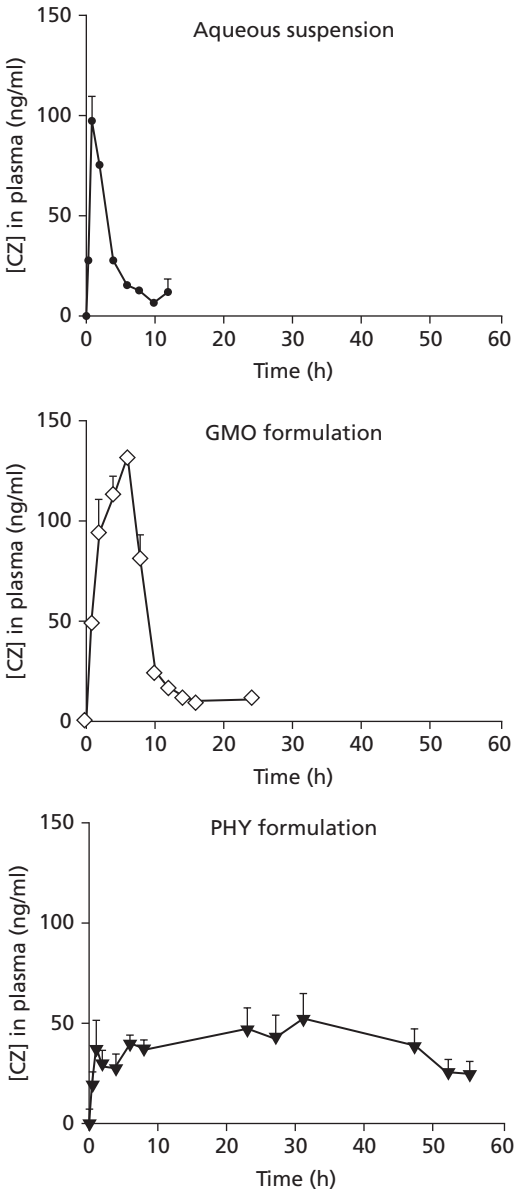

Figure 2 Dose-normalised plasma profiles after oral administration of cinnarizine. (●), CZ prepared as an aqueous suspension; (◇), as a lipid solution in glyceryl monooleate (GMO) and (▼), in phytantriol (PHY). Concentration of CZ in the vehicle = 7 mg/g, 300 mg of vehicle administered. (Data normalised to 7 mg/kg, $n = 4$, mean ± SEM).

rapid increase in CZ plasma concentration within the first 2 h post dose, and a higher C_{max} (134.0 ± 3.6 ng/ml) and BA (19.2 ± 0.3%) was calculated than for the suspension formulation. The T_{max} (5.0 ± 1.0 h) was also significantly longer ($P < 0.05$) than observed for the suspension (Table 3).

In contrast, CZ absorption from the PHY formulation was evident up to 55 h after administration. The plasma concentration of CZ stayed in the range 30–60 ng/ml between the 6 and 47 h time points, with T_{max} at 33.0 ± 5.0 h. A gradual fall in CZ plasma concentration followed and was below the quantifiable level in plasma after 60 h. Interestingly, the sustained plasma profile was unaffected by food intake, which was reintroduced 8 h after dosing. Although C_{max} was lower than that determined for the suspension formulation, the long duration of drug absorption resulted in a seven- to eight-fold increase in BA when compared to the suspension (Table 3).

Table 3 Mean pharmacokinetic parameters after oral administration of cinnarizine in suspension and phytantriol and glyceryl monooleate vehicles

Formulation	Mean C_{max} (ng/ml)	Mean T_{max} (h)	$AUC_{0\text{-t last}}$ (ng/ml.h)	F%
Suspension	96.9 ± 13.2	1.0 ± 0	313.5 ± 42.7	5.7 ± 0.8
GMO	134.0 ± 3.6	5.0 ± 1.0	1045.3 ± 15.5	19.2 ± 0.3
PHY	56.0 ± 11*	33.0 ± 5.0*	2210.2 ± 388*	40.5 ± 7.1*

Plasma concentrations were normalised to an equivalent dose of 7 mg/kg cinnarizine dosed per rat. Approximately 300 mg of lipid vehicle was administered per rat. GMO, glyceryl monooleate; PHY, phytantriol. Data expressed as mean ± SEM, $n = 4$, AUC was truncated at last sample. *$P < 0.05$ when compared to the suspension and GMO formulations.

Location of phytantriol and glyceryl monooleate formulations along the gastrointestinal tract after oral administration

PHY and GMO formulations (300 mg) were retained in the rat stomach for extended periods after oral administration (Figure 3). When the stomach was dissected at early times it was noted that a viscous mass resembling Q2 LC material was present in the proximal region of the stomach in the case of both PHY and GMO.

The PHY formulation was retained in the stomach for significantly longer than the GMO formulation (Figure 3). The similar proportions of CZ to PHY suggested that the drug remained associated with the lipid vehicle throughout the sampling period. A total of 67 ± 4.5% of the PHY formulation was retained in the stomach at 8 h and 56 ± 2.8% still remained after 24 h. The significant gastric retention of the PHY formulation was reflected in the small quantities of PHY and CZ recovered (<4%) in the intestine and faeces over 24 h (Table 4). However, the gastrointestinal recovery of the formulation after 24 h could not account for 44% of PHY and 60% of CZ, suggesting both drug and lipid were absorbed or degraded.

In contrast, the GMO formulation was poorly retained in the GIT relative to PHY. Only 66% of GMO remained in the stomach after 1 h. The quantity of CZ recovered was close to the quantity originally dosed, suggesting that the CZ had concentrated in the remaining lipid over time. At 8 h, less than 1% of the formulation remained in the stomach, with no detectable GMO present at 24 h. No lipid or drug were recovered from the GIT or faeces at 24 h, suggesting the formulation underwent extensive digestion and/or absorption in the GIT.

Absorption of phytantriol into the systemic circulation

Analysis of plasma from rats dosed with the micellar dispersion containing PHY indicated that PHY was readily absorbed from the GIT, with the quantity in plasma increasing consistently over 8 h (Figure 4). Hence, the

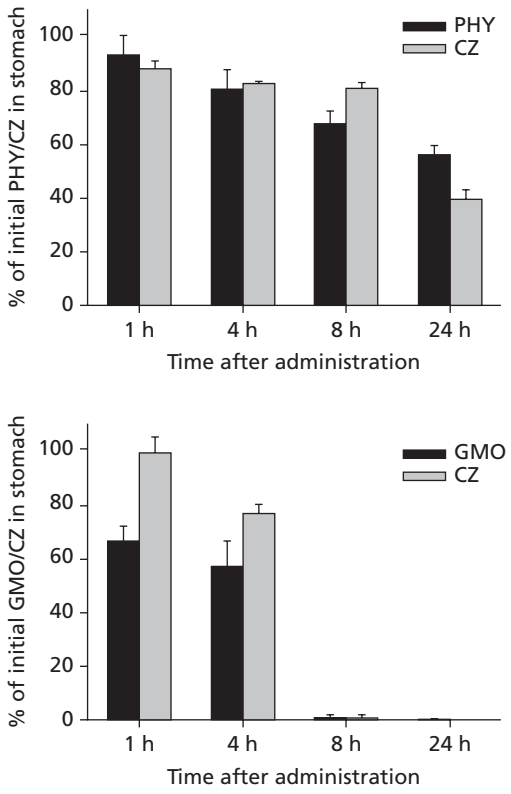

Figure 3 Cinnarizine and lipid remaining in the stomach against time after formulation administration. The percentage of cinnarizine (CZ) and lipid remaining in the stomach over time after oral administration of 300 mg of the phytantriol (PHY) formulation (top) and glyceryl monooleate (GMO) formulation (bottom). Values are mean ± SEM, $n = 3$

absorption of PHY in part explains the lack of recovery during the gastric retention studies, although the detailed pharmacokinetic behaviour is yet to be studied.

In-vitro assessment of digestion of phytantriol and glyceryl monooleate

The titration profiles resulting from the digestion of GMO and PHY are presented in Figure 5. The enzymatic digestion of 1 g of dispersed GMO resulted in approximately 64% digestion where 1 mole NaOH = 1 mole oleic acid. Not surprisingly, the exposure of dispersed PHY to enzymes did not result in an increase in pH as there is no process by which the liberation of fatty acids or any other pH-altering moieties can occur with the PHY molecule. Interestingly, the blank-subtracted profile for PHY indicated a slight decrease in liberation of fatty acid over time rather than an increase. This can be understood based on the fact that the blank micellar solution in which the particles are prepared consumes a finite amount of titrant because of lipolysis of the phospholipid present in micellar formulation. The current data therefore suggest that the presence of PHY may inhibit this lipolysis, leading to a negative consumption of titrant after subtraction of the micelle-only titration curve. A detailed understanding of this phenomenon is not clear at this time, but it may be that a proportion of the phospholipid associates with the PHY particles, preventing access by the

Table 4 The proportions of phytantriol (PHY), glyceryl monooleate (GMO) and cinnarizine recovered in different sections of the gastrointestinal tract after the oral administration of 300 mg of phytantriol and glyceryl monooleate formulations

		Recovery of formulation components (%)															
		Stomach		Duodenum		Jejunum		Ileum		Remaining intestine		Faeces		Total	
	Time	Lipid	CZ	Lipid	CZ	Lipid	CZ	Lipid	CZ	Lipid	CZ	Lipid	CZ	Lipid	CZ
PHY 300 mg	1 h	93 ± 7.2	87 ± 3.2	0.1 ± 0	0.4 ± 0.2	0.2 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.9	3.6 ± 0.2	0 ± 0	nd	**93 ± 7.1**	**88 ± 3.4**
	4 h	80 ± 6.9	82 ± 0.8	0.6 ± 0.2	0.2 ± 0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0	0.2 ± 0.1	1.0 ± 0.8	0 ± 0	nd	**81 ± 7.0**	**82 ± 0.8**
	8 h	67 ± 4.5	80 ± 1.9	–	–	–	–	–	–	–	–	–	–	–	–
	24 h	56 ± 3.4	39 ± 3.3	0.1 ± 0	0.1 ± 0	0.2 ± 0.1	0.1 ± 0	0.2 ± 0.1	nd	0.7 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.2 ± 0.1	**56 ± 2.8**	**40 ± 3.5**
GMO 300 mg	1 h	66 ± 5.4	99 ± 5.7	0.1 ± 0	0.2 ± 0	0.2 ± 0.1	1.2 ± 0.4	0.1 ± 0	0.2 ± 0.1	0.2 ± 0.1	1.2 ± 0.4	0.1 ± 0	0.2 ± 0.1	**67 ± 5.4**	**100 ± 5.6**
	4 h	57 ± 9.5	76 ± 3.6	0.1 ± 0	nd	0.1 ± 0	0.2 ± 0.1	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.2 ± 0.1	0.1 ± 0	0.1 ± 0	**57 ± 9.5**	**1 ± 0.1**
	8 h	0.9 ± 0.8	0.9 ± 0.8	–	–	–	–	–	–	–	–	–	–	–	–
	24 h	0.1 ± 0	0.9 ± 0.8	0.1 ± 0	nd	0.1 ± 0	nd	0.1 ± 0	nd	0.1 ± 0	nd	0.1 ± 0	nd	**0.3 ± 0**	**0 ± 0**

Values are mean ± SEM, $n = 3$; CZ, cinnarizine; GMO, glyceryl monooleate; PHY, phytantriol; nd, not detected.

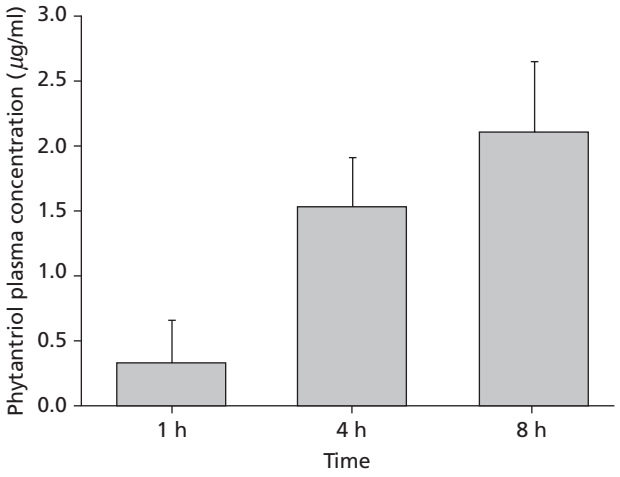

Figure 4 Phytantriol concentration in plasma after administration of mixed micelles. Concentration of phytantriol (PHY) measured in the plasma after the oral administration of 90 mg of PHY solubilised in mixed micelles (5 mM PC; 20 mM BS). Plasma samples were obtained 1, 4 and 8 h after oral administration in rats (mean ± SEM, $n = 3$). Lipid and drug concentrations were assayed using a validated HPTLC method.

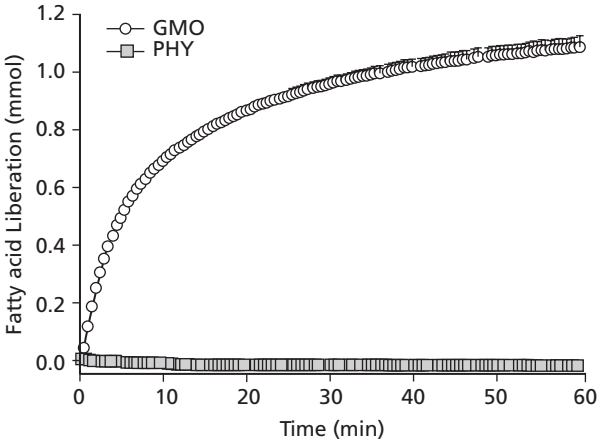

Figure 5 Fatty acid production over time on exposure of phytantriol and glyceryl monooleate dispersions to lipolytic enzyme. Fatty acid production, interpreted from titrant consumed by the pH stat, over time on exposure of glyceryl monooleate (GMO) and phytantriol (PHY) dispersions to lipolytic enzyme at 37°C. ($n = 3$, mean ± SD). Blank digestions of simulated intestinal fluid (SIF) were performed and the volume of titrant subtracted from the total to allow for fatty acids liberated by digestion of phosphatidyl choline in SIF.

lipolytic enzymes in the pancreatic extract. This is supported by the SAXS data reported in the accompanying paper,[6] in which association of model bile components with the PHY cubic structure was proposed as the mechanism, leading to increased lattice parameter with increasing bile concentration. Alternatively, it is possible that the Pluronic F127 included to facilitate dispersion of the lipids inhibited the action of the lipase, although this did not seem to be the case with GMO. Further work is required to discriminate these two possible effects.

HPTLC analysis of PHY before and after in-vitro digestion revealed no difference in the densitometric intensity or migration distance of the PHY band, further suggesting that the PHY molecule was not degraded or modified during exposure to digestive enzymes.

Discussion

In this study, the duration of systemic exposure to CZ from the PHY and GMO formulations was found to be dramatically increased when administered in PHY as compared to GMO. The prolonged exposure to CZ from cubic phase LCs has been observed after intraduodenal administration of medium chain length lipid formulations in the rat.[12] However, in that study the cubic phase system was a 'normal' phase system, where water is the continuous medium and the cubic phase comprises close-packed 'normal' micelles with hydrophobic tails on the inside, and hence is subject to phase change on dilution in gastrointestinal fluids. In the current study, the structures are 'inverse' phase liquid-crystal systems with a continuous bilayer structure, twisted and contorted on itself, which hence can co-exist in excess gastrointestinal fluids and therefore does not undergo phase transitions under the influence of dilution alone. It was therefore uncertain whether the prolonged exposure of CZ observed by Kossena *et al*. could be translated to orally administered formulations of CZ in PHY and GMO.

Previous studies using hydrophilic drugs have also demonstrated the ability of PHY and GMO cubic phases to control the release and absorption rate of drugs.[18] However, the duration of absorption in that case was only several hours, compared to the longer duration of absorption seen in the current study. More relevant behaviour has been observed on administration of CZ in a lipid that is a GMO analogue: oleyl glycerate.[7] This lipid, which forms an inverse hexagonal phase rather than a cubic phase in excess water, provided a similarly prolonged duration of absorption for CZ as the phytantriol formulation in the current study. Hence the formation of the liquid-crystal structure appears to be a factor in the behaviour. Administration of an oleic acid emulsion containing CZ to beagle dogs, in comparison to the current study, did not provide a sustained-release effect.[19] It is not clear what role particle size may play, as the formulations in the current study were administered as a lipid bolus. It is apparent that several different and related factors are likely to influence the sustained drug absorption for poorly water-soluble drugs using poorly digested LC systems, including: (1) the formation of a viscous sustained-release matrix, (2) delayed gastric emptying of the LC and (3) resistance of the LC to digestive processes.

Sustained release

The sustained absorption phase dominating the plasma profile for the PHY formulation is consistent with slow diffusion controlled CZ release from the gastrically retained LC. However, the very low solubility of CZ in gastrointestinal content, and retention of the formulation in the stomach for extended periods of time, may also indicate that the stomach is acting as a closed non-sink condition. CZ

must be absorbed or emptied from the stomach before more CZ can partition out of the formulation. It does not appear possible to discriminate between these two possibilities on the basis of this study. The close correlation between PHY and CZ concentrations over time suggests that a non-sink condition dominates, with slow erosion of the PHY Q_2 LC and emptying of particles from the stomach (Figure 4) possibly providing the means of absorption of CZ from the small intestine.

Gastric retention and digestibility

The limited gastric retention of the GMO formulation suggests that the LC structure may be degraded in the stomach. Lipase catalyses the hydrolysis of the ester functional group at the *sn1* or *sn3*-position of the acyl glycerol structure, releasing fatty acid and, as such, is expected to alter molecular packing within the LC. Previous studies have noted that GMO-based Q_2 LC systems are degraded when exposed to human, rat,[20] porcine[7] and bacterial[21,22] lipase enzymes, and undergo phase transitions from $Q_2 \rightarrow H_2 \rightarrow L_2$ phase. Lipase activity in the stomach is, however, relatively low, especially for long-chain glycerides,[23] and, as such, acid-catalysed hydrolysis (in addition to enzymatic lipolysis) may also be a significant contributor to degradation in the gastric environment, although not directly measured in this study. The L_2 phase formed on hydrolysis has very low viscosity, and its formation would be expected to accelerate gastric emptying (and therefore reduce the sustained-release effect) compared to the more viscous Q_2 and H_2 LC phases. The lack of a significant sustained-release effect with the GMO formulation could be expected based on these points.

The slightly longer T_{max} for CZ observed after administration of the GMO formulation (relative to the suspension) accorded with the gastric retention of the majority of the formulations for greater than 4 h after administration (Figure 3). Long-chain lipids have been shown to reduce gastric emptying[24] and previous studies using gamma scintigraphy have noted that the gastric retention of GMO exceeded the usual human gastric emptying times of 2–3 h,[25] with intact GMO remaining up to 6 h after ingestion.[26] It is not clear at this stage whether the presence of a liquid crystalline structure or lipid-induced gastric retention is the key to the delayed T_{max} for the GMO formulation. However, its ability to extend the duration of drug absorption in any case is much shorter than that of PHY.

The less-readily digested GMO analogue oleyl glycerate (which forms H_2 LCs) was found to dramatically sustain the absorption of CZ (seven-fold increase in T_{max}) when compared to GMO after oral administration to rats.[7] Perhaps coincidently, the T_{max} observed from the absorption of CZ from oleyl glycerate H_2 LCs was approximately the same as from the PHY Q_2 observed in this study (Figure 2). Although T_{max} is unlikely to directly correlate with susceptibility to digestive processing, the data from the two studies collectively indicate that poor digestibility is certainly one requirement for the observation of the absorption effect.

The sustained absorption of CZ from the PHY Q_2 LC was observed for an additional 47 h after the recommencement of feeding at 8 h. Given the prolonged gastric retention of the PHY Q_2 LC, it was interesting to note that the rats were readily eating and defecating, raising questions as to how the PHY LC resists postprandial gastric processing. Examination of the stomach after administration of the PHY formulation revealed the LC resided in the proximal region of the stomach, which is known as a reservoir for ingested material.[27] As such, the Q_2 LC may be somewhat protected from incoming food whilst residing in this gastric region, facilitating both sustained drug release and slow erosion of the matrix.

Absorption of phytantriol

Lastly, absorption of formulation components is commonplace in lipid-based formulations, where digestion of the lipids produces fatty acids and monoglycerides, which are in turn absorbed, in the same manner as regular dietary lipids. Analysis of plasma after oral administration of a dispersed PHY mixture revealed that PHY was readily absorbed into the systemic circulation (Figure 4), providing a rationale for the poor recovery of PHY from the different regions of the GIT during gastric retention studies (Table 4). Structurally similar compounds to PHY (i.e. those sharing a phytanyl chain such as α-tocopherol (vitamin E),[28] phytols[29–31] and phytanic acid[30]) have also been reported to be readily absorbed from the intestine. The pharmacokinetics and toxicological aspects of oral ingestion of PHY are yet to be studied. However, a recent report on the safety of PHY in cosmetic products reported that it did not induce aberrations in cultured human lymphocytes when tested within cytotoxicity limits, nor was it mutagenic in Ames tests, with or without metabolic activation, and it was neither phototoxic nor photoallergenic.[32] The mechanism of absorption and the effect of co-absorption of PHY on the sustained absorption of CZ remain uncertain, but warrant further investigation.

Conclusions

In this study PHY has been demonstrated as being capable of dramatically sustaining the absorption of a poorly water-soluble drug. Prolonged retention in the stomach, in part due to the non-digestible nature of the PHY allowing persistence of LC phase structure in the stomach, and non-sink conditions in the gastric compartment are believed to be the major contributors to this effect. In contrast, the rapid digestion of GMO is reflected in limited gastric retention, resulting in a lack of sustained absorption *in vivo*. The role of physicochemical factors, such as particle size and mucoadhesion, on gastric retention needs to be further explored, as does the applicability of the effect for other poorly water-soluble drugs and the effects in larger species. Nevertheless, this study has been the first to demonstrate the ability of the PHY cubic phase system to sustain the absorption of poorly water-soluble drugs *in vivo* and potentially opens the way for such formulations to be used as sustained oral drug-delivery systems.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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