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RESEARCH PAPER

# The Role of the Intestinal Lymphatics in the Absorption of Two Highly Lipophilic Cholesterol Ester Transfer Protein Inhibitors (CP524,515 and CP532,623)

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## ABSTRACT

***Purpose*** To evaluate the potential role of intestinal lymphatic transport in the absorption and oral bioavailability of members of an emerging class of anti-atherosclerosis drugs (CETP inhibitors). CP524,515 and CP532,623 are structurally related with $eLogD_{7.4}$ >5; however, only CP524,515 (and not CP532,623) had sufficient solubility (>50 mg/g) in long-chain triglyceride (LCT) to be considered likely to be lymphatically transported.

***Methods*** CP524,515 and CP532,623 were administered intravenously and orally to fasted or fed lymph-cannulated or non-cannulated dogs. Oral bioavailability and lymphatic transport of drug (and triglyceride) was subsequently quantified.

***Results*** Both CETP inhibitors were substantially transported into the lymphatic system (>25% dose) in fed and fasted dogs. Food enhanced oral bioavailability (from 45 to 83% and 44 to 58% for CP524,515 and CP532,623, respectively) and the proportion of the absorbed dose transported via the lymph (from 61 to 86% and from 68 to 83%, respectively). Lymphatic triglyceride transport was significantly lower in fed dogs administered CP532,623.

***Conclusion*** Intestinal lymphatic transport is the major absorption pathway for CP524,515 and CP532,623, suggesting that a LCT solubility >50 mg/g is not an absolute requirement for lymphatic transport. The effect of CP532,623 on intestinal lipid transport may suggest a role in the activity/toxicity profiles of CETP inhibitors.

**KEY WORDS** absorption · CETP inhibitor · intestine · lymphatic transport · torcetrapib

## ABBREVIATIONS

| | |
|---|---|
| CETP | cholesterol ester transfer protein |
| CM | chylomicron |
| HDL | high density lipoprotein |
| MTP | microsomal TG transfer protein |
| PLTP | phospholipid transfer protein |
| SMEDDS | self micro-emulsifying drug delivery system |
| TBME | *tert*-butyl methyl ether |
| TG | triglyceride |
| VLDL | very low density lipoprotein |
| LCT | long chain triglyceride |

## INTRODUCTION

Cholesteryl ester transfer protein (CETP) is present in the systemic circulation in association with high density lipoprotein (HDL) and mediates cholesterol ester transfer from HDL to lower density lipoproteins in exchange for triglyceride (TG) (1,2). Patients with certain CETP genotypes have elevated HDL cholesterol, a condition associated with a reduced risk of coronary heart disease (3,4). CETP inhibitors have therefore stimulated considerable interest as a potentially novel class of anti-atherosclerotic agents (1,5–7). The CETP inhibitors evaluated in the current study, CP524,515 and CP532,623, belong to a structural series of

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compounds which includes torcetrapib, a CETP inhibitor which showed considerable promise in early clinical trials but was eventually withdrawn from development in 2006 when phase III studies found an increased risk of mortality in patients receiving a combination of atorvastatin and torcetrapib rather than atorvastatin alone (8). The reason for increased mortality in the group receiving torcetrapib appears to be related to an off-target effect leading to increases in blood pressure, rather than inhibition of CETP. As such, a number of companies continue to pursue CETP inhibition as a strategy to treat dyslipidemia (9).

The physicochemical profile of the torcetrapib series of CETP inhibitors (10) is typical of many modern drug candidates, in that they are highly lipophilic and poorly water soluble. High lipophilicity and low water solubility may lead to reduced drug absorption and bioavailability after oral administration, and compounds of this type often fail to progress into formal development (11). However, recent advancements in the design of delivery systems that promote intestinal solubilisation, including lipid-based formulations (12,13) and solid dispersions (14), have shown that reproducible absorption of lipophilic and poorly water soluble drug candidates can be achieved and that clinical development of highly lipophilic drug candidates is both possible and increasingly common.

Following oral delivery, most drugs are absorbed across the intestinal absorptive cells (enterocytes) and transported to the systemic circulation via the portal blood. For highly lipophilic drugs, however, drug association with lipoprotein assembly pathways in the enterocyte may lead to the transport of a proportion of the absorbed dose to the systemic circulation via the intestinal lymph (15). Lymphatic transport of lipophilic drugs is enhanced by administration with fatty meals (16) or lipidic formulations (17), since ingested lipids stimulate the formation of TG-rich lipoproteins in the enterocyte (18). The extent of lymphatic drug transport is therefore dependent on a supply of lipoprotein precursors in the enterocytes and on the propensity of a drug to associate with lipid transport pathways on passage across the enterocyte.

A number of studies have described simple predictors of the potential for substantial lymphatic drug transport. Charman and Stella first proposed that a log P >4.7 and long-chain TG solubility >50 mg/g were pre-requisites for drugs to exhibit significant intestinal lymphatic transport (19). Recently, a more complex in silico method has been described in order to better predict the likelihood of lymphatic drug transport based on simple molecular descriptors (20). Gershkovich *et al.* have also suggested that ex-vivo drug solubility in plasma TG-rich lipoproteins can be used to estimate the extent of drug transport via the lymphatic system (21) and have described an in silico model to predict drug association with TG-rich lipoproteins (22) and therefore lymphatic drug transport. As part of the latter study the authors further noted that drug $LogD_{7.4}$, rather than LogP, had the strongest correlation with drug affinity for TG-rich lipoproteins and, likely, therefore lymphatic drug transport (22). A common requirement for lymphatic transport across all of these models, therefore, is appreciable drug lipophilicity.

The increasingly frequent development of drug candidates with high lipophilicity suggests that intestinal lymphatic transport may play a growing role in drug absorption and disposition profiles. Historically, the significance of intestinal lymphatic drug transport has been thought to lie in the possibility of a reduction in first-pass hepatic metabolism since the lymphatic system, unlike the portal vein blood, empties directly into the systemic circulation and effectively by passes the liver. However, entry into the systemic circulation in association with TG-rich lipoproteins may also alter systemic drug distribution patterns (23,24) and might therefore be expected to influence drug toxicity and efficacy profiles. This may be particularly important for compounds such as CETP inhibitors where drug association with TG-rich lipoproteins (very low density lipoproteins (VLDL) and chylomicrons (CM)) in the lymph and delivery to the systemic circulation in association with lipoproteins might be expected to influence their capacity to alter lipid exchange between lipoproteins. Previous studies have also shown that relatively limited changes to formulation components or the co-administration of food may markedly affect the extent of lymphatic drug transport (11,16,17). The need for an early indication of the potential for intestinal lymphatic transport to play a role in the biopharmaceutical profile of a new drug candidate or indeed a class of drug candidates, such as CETP inhibitors, is therefore apparent.

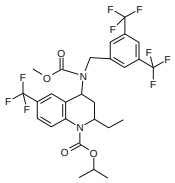
The current study has examined the effect of food on the lymphatic transport and oral bioavailability of the CETP inhibitors CP524,515 and CP532,623 in greyhound dogs. CP524,515 and CP532,623 are structurally similar (see Table I) and are highly lipophilic as assessed by distribution co-efficient ($eLogD_{7.4}$ >5.5). However, the lipid solubility of CP532,623 is considerably lower than that of CP524,515 and lower than that historically ascribed by Charman and Stella (19) to compounds which are significantly lymphatically transported. Comparison of the extent of absorption and lymphatic transport of the structurally related compounds CP524,515 and CP532,623 therefore provides a unique opportunity to more closely probe the physicochemical requirements for lymphatic transport.

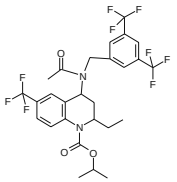
## MATERIALS AND METHODS

### Materials

CP524,515 and CP532,623 (Pfizer Inc, Groton, CT), Miglyol 812 (Sasol, Germany), Maisine 35-1 (Gattefosse, France),

**Table I** Physical and Chemical Properties of CP524,515 and CP532,623





| | CP524,515 | CP532,623 |
|---|---|---|
| Molecular weight (Da) | 614.51 | 598.51 |
| cLog P[a,b] | 7.86 / 7.55 | 6.62 / 6.42 |
| Elog $D_{3.0}$[c] | **6.07** | **5.63** |
| Elog $D_{7.4}$[c] | **6.13** | **5.68** |
| Melt Onset[b] | 76.0°C | 101.8°C |
| Miglyol solubility (mg/ml)[d] | 164.9 ± 0.9 | 61.2 ± 0.9 |
| Soybean oil solubility (mg/ml)[d] | 50.9 ± 0.1 | 16.4 ± 0.4 |

[a] ISIS Base 2.3® and ACD/Labs 4.56 calculations respectively

[b] Data on file, Pfizer Global Research and Development, Groton, CT

[c] Determined by modification of the method by Lombardo *et al.* (25)

[d] Mean ± SEM, $n=4$

Intralipid (Baxter, Australia), N,N-dimethylacetamide (Prolabo, France), ammonium acetate and formic acid (Fluka, Sigma-Aldrich, Germany) were used as received. Chloroform, methanol, acetonitrile, ethanol and *tert*-butyl methyl ether (TBME) were obtained from Merck, Germany and were of analytical grade. Di-potassium and di-sodium EDTA, soybean oil, Cremophor EL, and triacetin were obtained from Sigma-Aldrich, St Louis, MO.

## Triglyceride Solubility Studies

100 mg of CP524,515 or CP532,623 and 500 mg of soybean oil (long-chain TG) or Miglyol (medium-chain TG) were weighed into a glass vial. The samples were vortexed for 5 min and the mixtures were incubated at 37°C for 1 h then returned to room temperature. Samples were also vortexed for 10 s every 2–3 h. At 6, 24, 48 and 72 h the mixtures were vortexed for 10 s, centrifuged at 400 g for 20 min to precipitate undissolved drug and 1 drop of the resulting supernatant was weighed directly into a 5 ml volumetric flask taking care not to disturb the drug pellet. 50 µl of 1 M HCl was added to the samples and the volumetric flask made up to 5 ml with 2:1 *v/v* chloroform/methanol. 10 µL of the chloroform/methanol solution was subsequently diluted 1:10 *v/v* with acetonitrile and the drug concentration measured by HPLC. The solubilities of CP524,515 and CP532,623 are reported in Table I and reflect equilibrium solubility measurements (defined as the point at which solubility measurements obtained for subsequent samples differed by less than 5%).

## Chromatographic LogD Estimation Method

Distribution coefficients of the test compounds were estimated at pH 3.0 and 7.4 using a variation of the methods described by Lombardo *et al.* (25) and Donovan *et al.* (26) that correlates known partition coefficient values of drug molecules with HPLC retention characteristics on a reverse phase column. The set of standard compounds, mobile phase composition and flow rate and HPLC column were modified in this method when compared to those described previously. In brief, a series of standard compounds with known distribution coefficient values (17-hydroxyprogesterone, chloroquine, coumarin, hydrocortisone-21-acetate, ketoconazole, metronidazole, nadolol, probenecid, pyrene, theophylline, tolnaftate) were prepared as 100 µg/mL solutions in methanol and the test compounds (CP532,623 and CP524,515) were prepared as 25 µg/mL solutions in 50% acetonitrile/water. The compounds were then separated chromatographically using an HPLC system consisting of a Waters 2795 alliance system, Waters 2487 dual channel UV/visible detector controlled via Waters empower software (Waters Corp., Milford, MA). 10 µL samples of the standard compounds and CP532,623 and CP524,515 were injected onto a Phenomenex Synergi Hydro-RP® 4 µm (30×2 mm) column (Phenomenex, Australia) at 25°C. The mobile phase flow rate was 0.4 mL/min and the mobile phases consisted of acetonitrile and buffer (50 mM ammonium acetate at pH 7.4 for LogD determination at pH 7.4 and 50 mM ammonium formate at pH 3.0 for LogD determination at pH 3.0). The gradient sequence was initiated at 100% buffer which was linearly increased to 100% acetonitrile over 10 min, held at 100% acetonitrile for 0.5 min, returned to 100% buffer over 0.5 min and held at 100% buffer for 2.5 min. The total run time was 13.5 min. The retention time of the standard compounds correlated linearly with their known LogD at pH 3.0 and pH 7.4. The $LogD_{3.0}$ and $LogD_{7.4}$ of CP532,623 and CP524,515 were calculated from their retention times and comparison to the standard curve.

## Animal Studies

Adult male greyhound dogs (27–39 kg) were used in all studies. Surgical procedures were approved by the local animal ethics committee and performed in accordance with the Australian and New Zealand Council for the Care of Animals in Research and Teaching guidelines as described previously (16,27).

## Design of Animal Studies

The contribution of lymphatic transport to the fasted and post-prandial bioavailability of CP524,515 and CP532,623 was examined using three separate animal studies. In the

first study, plasma and lymph samples were taken following oral administration of CP524,515 or CP532,623 with a long-chain lipid based formulation to fasted or fed lymph-cannulated greyhound dogs. These studies enabled determination of the extent of drug absorption into the systemic circulation via the intestinal lymphatic system and portal vein in fasted or post-prandial lymph-cannulated dogs. In the second study, the same formulations were similarly administered to fasted and fed non-lymph cannulated greyhound dogs (ie where the lymph duct remained intact) and plasma samples taken to allow assessment of total systemic exposure to CP524,515 and CP532,623. In the final study, CP532,623 and CP524,515 were administered via intravenous (IV) bolus to non-lymph cannulated greyhound dogs to allow subsequent assessment of the oral bioavailabilities of CP524,515 and CP532,623.

## Formulation Preparation

### *Oral Studies*

Long-chain lipid based self micro-emulsifying drug delivery systems (SMEDDS) were prepared according to previously established methods (16). The SMEDDS formulation consisted of 30.5% *w/w* soybean oil, 30.5% *w/w* Maisine 35-1, 31.6% *w/w* Cremophor EL and 7.4% *w/w* ethanol. For administration of CP524,515, dogs were dosed with one × 1 g capsule containing 50 mg CP524,515 dissolved in 950 mg of the SMEDDS formulation. For administration of CP532,623, dogs were dosed with two × 1 g capsules containing a total dose of 50 mg CP532623 dissolved in 1,950 mg of the SMEDDS formulation. CP532,623 was dosed in two × 1 g capsules as the solubility of CP532,623 was insufficient to allow incorporation of the 50 mg dose into 950 mg of the SMEDDS. Formulations were filled into soft gelatin capsules using a 3 mL syringe and 18 g needle and the capsules sealed using a warm spatula. The stability, dispersion and emulsification characteristics of the oral formulations were assessed as described previously (16).

### *Intravenous Studies*

CP532623 and CP524515 were incorporated into an intralipid emulsion using an adaptation of a previous method (28). Briefly, 12 mg of either CP532623 or CP524515 was dissolved in 320 μL N,N dimethylacetamide:triacetin (3:5 *v/v*) and the resultant mixture added dropwise (5 μL per addition) to 15 mL of intralipid. The intralipid formulation was ultrasonicated between each dropwise addition of drug using a Misonix XL 2020 ultrasonic processor (Misonix, Farmingdale, NY) equipped with a 3.2 mm microprobe which was pulsed (1 min on/20 s off) at an amplitude of 240 μm and a frequency of 20 kHz. During ultrasonication, the emulsion was cooled on ice to below 10°C. The emulsion was stored at 4°C overnight prior to dosing. Immediately prior to dosing, the emulsion was passed through a 0.2 μM filter (Minisart CE, Santorius, UK). An aliquot of the filtered emulsion was subsequently assayed for drug content by HPLC to accurately determine the dose administered.

## Lymphatic Transport Studies in Lymph-Cannulated Dogs

The extent of lymphatic transport of CP524,515 and CP532,623 was evaluated in fasted or fed lymph-cannulated male dogs ($n=3$ per group).

### *Surgical Procedures and Recovery*

The thoracic lymph duct was cannulated under surgical anaesthesia as previously described (27). Following surgery, dogs were allowed to recover unrestrained in a closed run overnight (12–16 h) and returned to normal ambulatory movement before commencement of the study. In the initial recovery period fluids were administered IV to ensure adequate hydration and to prevent hypoproteinemia (27). Water was also available *ad libitum*.

Prior to drug administration, a 20 Gauge intravenous catheter was inserted into the cephalic vein to enable serial blood sampling and the catheter kept patent by periodic flushing with heparinised saline (1 IU/mL). For treatment groups where formulations were administered to fasted animals, food was withheld throughout both the recovery and study periods. For fed-state (post prandial) studies, the dogs were fasted overnight prior to feeding a standard can of commercial dog food (680 g) containing 5% fat and 7.5% protein 30–45 min prior to drug administration. To limit possible dehydration due to the continuous collection of thoracic lymph, 25 mL normal saline was also administered hourly by IV bolus during the sampling period. Water was available *ad libitum* throughout the sample collection period. At the end of the sampling period, dogs were humanely killed via an intravenous dose of 200 mg/kg phenobarbitone (Jurox, Silverwater, Australia).

### *Experimental Procedures*

CP524,515 (1 × 1 g SMEDDS capsule containing 50 mg drug) or CP532,623 (2x1 g SMEDDS capsules containing 50 mg drug) formulations were administered to the fasted or fed greyhound dogs by placing the capsule as far posterior to the pharynx as possible, closing the mouth and rubbing the throat to stimulate swallowing. 50 mL of water was subsequently administered orally via a syringe. Lymph was collected continuously into pre-weighed 50 mL collection tubes containing 75 mg di-sodium EDTA for the duration of the

10 h post-dosing period. Individual lymph samples for each half hourly, hourly or 2 hourly collection period (up to 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 10 h post-administration of the drugs) were combined and the mass of lymph collected determined gravimetrically. Three separate 200 μL aliquots of each lymph sample were transferred into individual 1.5 mL eppendorf tubes and stored at 4°C until analysis of drug and TG concentrations (within 24 h). Systemic blood samples (3 mL) were obtained via the indwelling cephalic vein catheter into individual tubes containing di-potassium EDTA. Blood samples were collected at pre-dose (−5 min), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 10 h following drug administration. Plasma was separated by centrifugation and stored at −80°C prior to analysis by LC-MS.

## Pharmacokinetic and Oral Bioavailability Studies in Non-lymph-Cannulated Dogs

The absolute oral bioavailability of CP524,515 and CP532,623 was evaluated in non-lymph cannulated dogs by comparison with data obtained after intravenous administration. The oral data was generated as a randomised cross over study in $n=3$ greyhound dogs. The IV data ($n=3$) was generated in a separate cohort of animals. The washout period between drug administrations was at least 1 week in all cases. Prior to each study day, dogs were fasted overnight in a closed run (12–16 h) with free access to water and prior to dosing 20 Gauge intravenous catheters were inserted into the cephalic veins to enable collection of serial blood samples. Catheters were kept patent by flushing with heparinised saline (1 IU/mL). For fasted-state studies, food was withheld until 8 h post dose. For fed-state (post-prandial) studies, the dogs were fasted overnight and fed a standard can of commercial dog food (680 g) containing 5% fat and 7.5% protein 30–45 min prior to drug administration.

### *Oral Bioavailability Studies in Non-lymph-Cannulated Dogs*

CP524,515 (1×1 g SMEDDS capsule containing 50 mg drug) or CP532,623 (2x1 g SMEDDs capsules containing 50 mg drug) formulations were administered orally to fasted or fed greyhound dogs as described earlier. Systemic blood samples (3 mL) were collected into individual tubes containing dipotassium EDTA at 5 min prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8 and 24 h after dosing. Plasma was separated by centrifugation and stored at −80° prior to analysis.

### *Intravenous Pharmacokinetic Studies in Non-lymph-Cannulated Dogs*

In the intravenous studies, 5 mL of the intralipid-drug formulation (containing 4 mg drug) was administered by slow intravenous bolus injection into the cephalic vein catheter at a rate of 1 mL/min for 5 min. The catheter was then flushed with 5 mL of heparinised (1 IU/mL) saline. Systemic blood samples (3 mL) were obtained via the opposite indwelling venous catheter and collected into individual tubes containing di-potassium EDTA. Blood samples were collected 5 min prior to dosing and at 10, 20, 30, 40, 50, 60, 75, 90 min and 1, 2, 3, 4, 6 and 8 h after dosing. Plasma was separated by centrifugation and stored at −80° prior to analysis.

## Sample Preparation

### *Lymph Samples*

Lymph samples were prepared for HPLC analysis by dilution 1:40 *v*/*v* with acetonitrile, vortexing for 1 min and centrifuging for 3 min at 400 g. This dilution was sufficient to ensure quantitative recovery of CP524,515 and CP532,623 from lymph. The efficiency of the sample dilution strategy was validated by assessment of the recovery of drug from $n=3$ lymph samples spiked with known concentrations of CP524,515 and CP532,623 at low, medium and high concentrations (10, 20, 100 μg/mL).

### *Plasma Samples*

The concentrations of CP532,623 and CP524,515 in plasma were determined using a validated LC-MS method following extraction from plasma. 500 μL plasma samples were initially spiked with 200 ng/ml internal standard (CP532,623 was used as the internal standard for CP524,515 and vice versa) and vortexed for 1 min. 500 μl acetonitrile was subsequently added to precipitate proteins, samples vortexed for 1 min and centrifuged for 5 min at 400 g. 4 ml TBME was added and samples vortexed for a further 1 min then centrifuged for 5 min at 400 g. The supernatant was transferred into a separate 10 ml polypropylene tube and evaporated to dryness under a steam of high purity nitrogen at 35°C (N-EVAP 112 evaporator, Organomation, MA, USA). The residue was reconstituted in 100 μL of mobile phase B (see below). 5 μL was subsequently injected onto the LC-MS. The efficiency of extraction was greater than 85% for both CP532,623 and CP524,515 as assessed by the extraction of quality control samples ($n=3$ replicates) spiked with known drug at low, medium and high drug concentrations (2.5–1,000 ng/mL).

## Sample Analysis

### *HPLC*

The HPLC system consisted of a Waters 2690/5 Alliance module and Waters 486 tuneable absorbance detector (set

at a wavelength of 254 nm) controlled via Waters empower software (Waters Corp., Milford, MA). 20 μL samples were injected onto a 150×4.6 mm reverse phase C8 column with a pore size of 5 μm (Phenomenex Luna®, Phenomenex, Australia) fitted with a Phenomenex security guard RP-C8 precolumn (4×3 mm, Phenomenex, Australia). Mobile phase A was 10:90 (*v*/*v*) acetonitrile:milli-Q water and mobile phase B consisted of 95:5 (*v*/*v*) acetonitrile:milli-Q water. The mobile phase flow rate was 1 mL/min. The mobile phase gradient sequence was initiated at 50% mobile phase B, linearly increased to 100% mobile phase B over the next 8 min, prior to holding at 100% B for 5.5 min, and return to 50% B over 4.5 min. The total run time was 18 min and using this method, CP532,623 eluted at 10 min and CP524,515 eluted at 11 min.

The HPLC method for CP524,515 and CP532,623 were validated by assay of replicate ($n$=4) quality control samples at low, medium and high concentrations (0.2, 1, 5 μg/mL) on three separate days. The assays were found to be accurate (within 10% of target concentration) and precise (co-efficient of variation <10%) for concentrations between 0.2 and 10 μg/mL. Inter-day variability in precision and accuracy was <10% and the limit of quantitation was 200 ng/mL.

### *LC-MS*

The LC-MS system consisted of two LC-20AD pumps, an on-line DGU-20A5 solvent degasser, CTO-20A column oven and a single quadrupole mass spectrometer with an ambient pressure chemical ionisation (APCI) interface (Shimadzu, Kyoto, Japan). Data acquisition and processing were performed using LCMS Solutions software (Shimadzu Scientific Instruments, Kyoto, Japan). 5 μL samples were injected onto a Phenomenex Gemini C18 110A column (3 μm particle size, 50 mm×2.00 mm i.d. Phenomenex, CA) at 29°C and the mobile phase flow rate was 0.4 mL/min. Mobile phase A was 95:5 (*v*/*v*) water: methanol with 2 mM ammonium acetate and 0.1% formic acid, mobile phase B was 85:10:5 (*v*/*v*) methanol:water: acetonitrile with 2 mM ammonium acetate and 0.1% formic acid. The mobile phase gradient sequence was as follows: mobile phase B was initially held at 85% for 0.5 min, then linearly increased to 100% over the next 2.5 min, prior to holding at 100% for 2.5 min, and return to 85% over 0.5 min. Mobile phase B was then held at 85% for 3 min prior to injection of the subsequent sample. The total run time was 9 min per injection with the flow to the MS redirected to waste for the first 1.5 min and between 7 and 9 min using a flow diverter positioned after the column. CP532,623 eluted at 3.2 min and the CP524,515 eluted at 3.9 min.

LC-MS detection of CP532,623 and CP524,515 was effected by a Shimadzu single quadrupole mass spectrometer with an ambient pressure chemical ionisation (APCI) interface in positive mode. CP532,623 was detected by selective ion monitoring (SIM) of the 615.90 mass/charge ion peak ($m/z$) ($[M+H_2O]^+$) and CP524,515 at 631.80 $m/z$ ($[M+H_2O]^+$). The APCI interface was maintained at 250°C and the heat block and curved desolvation line (CDL) at 200°C. Interface and CDL voltages were 4.5 kV and −50.0 V respectively. The nebulizer gas flow rate was 2.5 L/min, and the drying gas flow rate 10 L/min.

The LC-MS methods for CP524,515 and CP532,623 were validated by assay of replicate ($n$=4) quality control samples at low, medium and high concentrations (2.5, 20, 200 and 500 ng/mL) on three separate days. The assays were found to be accurate (within 10% of target concentration) and precise (co-efficient of variation <10%) for concentrations between 2.5 and 1,000 ng/mL. Inter-day variability in precision and accuracy was <10% and the limit of quantitation was 2.5 ng/mL.

### *Lymph Triglyceride Analysis*

TG concentrations in lymph were measured using a Cobas Mira® lipid analyser (Roche Diagnostics, Indianapolis, IN) running a commercial enzymatic colorimetric reagent (Triglyceride kit®, Roche Diagnostics, Indianapolis, IN) as described previously (16).

## Data Analysis

### *Mass of Drug and Lipid Transported in Lymph*

The mass of CP532,623, CP524,515 and TG transported in lymph was calculated as the product of measured concentrations of CP532,623, CP524,515 and TG in lymph and the volume of lymph collected (as determined gravimetrically) for each collection period.

### *Pharmacokinetic Parameters Following Intravenous Dosing to Non-lymph-Cannulated Dogs*

First order terminal elimination rate constants (k) were determined from the gradient of the terminal log-linear phase of individual plasma drug concentration-time profiles and the elimination half life ($T_{1/2}$) subsequently calculated as 0.693/k. The area under the plasma concentration-time profiles from time zero to the last measured concentration ($AUC_{0\text{-}tz}$) was calculated using the linear trapezoidal method. The area under the plasma concentration-time profiles from time zero to infinity ($AUC_{0\text{-}\infty}$) was calculated by adding the area obtained by extrapolation from the last plasma sample ($Cp_{last}$) to infinite time ($Cp_{last}/k$) to $AUC_{0\text{-}tz}$.

Volume of distribution ($V_{Darea}$) and clearance (Cl) were calculated from:

$$Cl = D_{IV}/AUC_{0-\infty} \quad (1)$$

$$V_{Darea} = D_{IV}/(k.AUC_{0-\infty}) \quad (2)$$

where $D_{IV}$ is intravenous dose of CP524,515 or CP532,623.

### *Pharmacokinetic Parameters Following Oral Dosing to Lymph-Cannulated and Non-lymph-Cannulated Dogs*

The maximum plasma concentration ($C_{max}$), and the time to reach the maximum plasma concentration ($T_{max}$) were noted directly from the individual plasma drug concentration-time profiles following oral dosing to lymph-cannulated or non-lymph cannulated dogs. The first order terminal elimination rate constants (k) were determined from the gradient of the terminal log-linear phase of the individual plasma drug concentration-time profiles and the elimination half life ($T_{1/2}$) subsequently calculated as 0.693/k. The area under the plasma concentration-time profiles from time zero to the last measured concentration ($AUC_{0\text{-}tz}$) were calculated using the linear trapezoidal method. The area under the plasma concentration-time profiles from time zero to infinity ($AUC_{0\text{-}\infty}$) were calculated by adding the area obtained by extrapolation from the last plasma sample ($Cp_{last}$) to infinite time ($Cp_{last}/k$) to $AUC_{0\text{-}tz}$.

### *Oral Bioavailability in Non-lymph-Cannulated Dogs*

The oral bioavailability of CP524,515 and CP532,623 in non-lymph cannulated dogs were calculated as the ratio of the dose-normalised AUC values following oral and IV administration, ie:

$$F_{Total} = (AUC_{PO}/D_{PO})/(AUC_{IV}/D_{IV}) \cdot 100\% \quad (3)$$

where $D_{PO}$ and $D_{IV}$ are the total doses administered via the oral and intravenous routes, respectively, and $AUC_{PO}$ and $AUC_{IV}$ represent the $AUC_{0\text{-}\infty}$ in plasma following oral and IV dosing, respectively.

### *Absorption into the Lymph and Blood in Lymph-Cannulated Dogs*

The fraction of the dose of CP532,623 and CP524,515 absorbed via the intestinal lymphatic system ($F_{lymph}$) following oral administration to the lymph-cannulated dogs was calculated as the ratio of cumulative mass of CP532,623 or CP524,515 transported in the lymph and the oral dose. The proportion of the dose absorbed into the systemic circulation via the portal route ($F_{blood}$) following oral administration to lymph-cannulated greyhounds was estimated from the dose-normalised plasma $AUC_{0\text{-}\infty}$ for CP532,623 or CP524,515 following oral administration to lymph-cannulated greyhounds relative to the dose-normalised $AUC_{0\text{-}\infty}$ for CP532,623 or CP524,515 following IV administration to non-lymph cannulated dogs, i.e.

$$F_{blood} = \left(AUC_{lymph-cannulated}/D_{PO}\right)/(AUC_{IV}/D_{IV}) \cdot 100\% \quad (4)$$

where $D_{PO}$ and $D_{IV}$ are the total doses administered via the oral and intravenous routes, respectively, and $AUC_{lymph\text{-}cannulated}$ and $AUC_{IV}$ represent the $AUC_{0\text{-}\infty}$ in plasma following oral dosing to lymph-cannulated dogs and IV dosing to non-lymph cannulated dogs. The oral bioavailability of CP532,623 or CP524,515 in the lymph-cannulated greyhounds ($F_{total}$) was calculated from the sum of $F_{lymph}$ and $F_{blood}$.

## Statistics

Statistically significant differences were determined by ANOVA followed by Tukey's test for multiple comparisons at a significance level of $\alpha = 0.05$. All statistical analysis was performed using SPSS for Windows version 15.0 (SPSS Inc, Chicago, Il).

# RESULTS

## Physicochemical Properties

The results of the solubility studies and LogD estimations, together with selected physiochemical parameters of CP532,623 and CP524,515 are reported in Table I. The octanol-water partition and distribution co-efficients of CP524,515 and CP532,623 were high in both cases (cLog P >6.5, eLog $D_{7.4}$ >5) with LogP/LogD values being slightly higher for CP524,515 when compared with CP532,623. Both drugs were more soluble in medium chain TG (Miglyol) when compared with long chain TG (soybean oil), consistent with the view that drug solubility in oils is most highly correlated with the concentration of ester bonds in triglyceride and is therefore higher (on a mass per mass basis) in lower molecular weight glycerides (29). CP524,515 was highly soluble in both medium chain TG (165 mg/g) and long chain TG (50 mg/g). In contrast, CP532,623 was less TG soluble (with solubilities of 60 mg/g and 16 mg/g in medium and long chain TG respectively), consistent with its lower LogP and LogD and a lower melt onset temperature of 76°C for CP524, 515 when compared to CP532,623 (102°C).

## Intravenous Pharmacokinetic Studies

The plasma profiles of CP524,515 and CP532,623 declined bi-exponentially following intravenous administration

(Fig. 1). The pharmacokinetic parameters obtained are summarised in Tables II and III, respectively. The $V_{Darea}$ of both compounds were similar (1.5–2 L/kg), however the clearance of CP532,623 was significantly ($\alpha < 0.05$) greater than that of CP524,515, resulting in a slightly shorter terminal elimination half life (2.0 *versus* 3.9 h).

## Oral Bioavailability and Pharmacokinetic Studies in Non-lymph-Cannulated Dogs

The plasma concentration-time profiles obtained following oral administration of a SMEDDS formulation of CP524,515 to fed or fasted, non-lymph cannulated greyhound dogs are depicted in Fig. 2A and the summary pharmacokinetic parameters given in Table II. It is evident that systemic exposure to CP524,515 increased significantly when administered post-prandially (1.8 and 3.9 fold increases in $AUC_{0-\infty}$ and Cmax, respectively). $T_{max}$ also decreased slightly from a median of 1.5 to 1 h. Comparison with the IV profiles suggests an increase in the absolute bioavailability from 45% to 83% in fed *versus* fasted animals (Table IV).

Fig. 2C shows the plasma-concentration profile observed following oral administration of a SMEDDS formulation of CP532,623 to fed or fasted, non-lymph cannulated greyhound dogs and Table III provides a summary of the pharmacokinetic data obtained. Consistent with the absorption patterns for CP524,515, $AUC_{0-\infty}$ and $C_{max}$ increased significantly (1.3 and 2.4 fold, respectively) following post-prandial administration of CP532,623 and the $T_{max}$ occurred earlier (median 1.0 *versus* 2.0 h). Food significantly ($\alpha < 0.05$) enhanced the bioavailability of CP532,623 from 44.0% to 58% (Table IV), however, the increases in post-prandial bioavailability were not as large as those seen for CP524,515. Interestingly, the fasted oral bioavailability of CP532,623 was similar to that of CP524,515 and the reduced food effect was evident only in lower oral bioavailability after post-prandial administration. The terminal half-life of CP532,623 was apparently longer in the non-lymph cannulated dogs (8.5–11.9 h) administered CP532,623 via the oral route when compared to the lymph-cannulated dogs administered CP532,623 via the oral route (3–3.8 h) and dogs administered CP532,623 intravenously (2 h). However, the relatively sparse data in the terminal phase dictated that the estimates of the terminal rate constant were highly dependent on a single (24 h) plasma sample. As such the differences obtained may well reflect variability in quantification of the 24 h plasma sample (which was close to the LOQ of the assay (5 ng/mL)) rather than a true change to disposition. Importantly, the extrapolated AUC's represent a small proportion of the total AUC and therefore any variability in terminal half life estimation had a minor impact on bioavailability estimates.

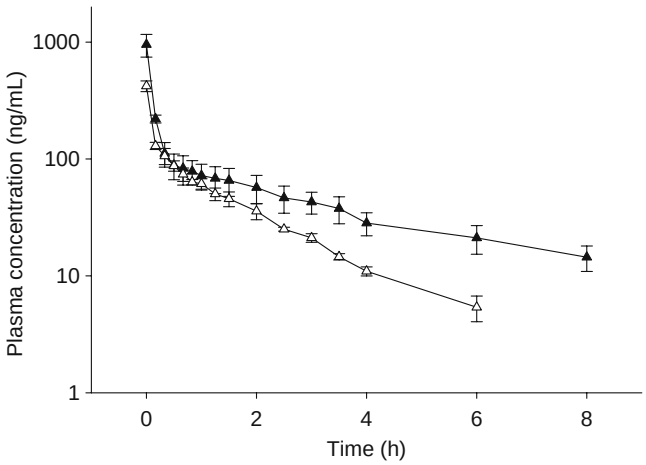



**Fig. 1** Systemic plasma concentration-time profiles after intravenous administration of 4 mg of CP532,623 (Δ) or CP524,515 (▲) as an intralipid emulsion to non-lymph-cannulated, fed greyhound dogs. Data presented as mean ± SEM for $n = 3$ dogs.

## Oral Bioavailability, Lymphatic Transport and Pharmacokinetic Studies in Lymph-Cannulated Dogs

The cumulative extent of drug transport into the lymph and the rate profiles describing lymphatic drug transport as a function of time following oral administration of CP524,515 and CP532,623 to fed or fasted lymph-cannulated dogs are given in Figs. 3 and 4, respectively. Table IV summarises the proportion of the administered dose that was recovered in lymph ($F_{lymph}$) and an estimate of the proportion absorbed directly into the blood ($F_{blood}$). $F_{blood}$ was calculated from the dose-normalised plasma AUC in lymph-cannulated animals relative to the AUC after IV administration to non-lymph cannulated animals. The cumulative recovery of CP524,515 in the intestinal lymph over a 10 h post-dose period following administration of a single 1 g capsule of SMEDDS represented a substantial proportion of the dose (25.2%) and the extent of lymphatic transport increased further to 56.0% of the dose on administration to fed animals (Table IV). The maximum rate of CP524,515 transport into lymph (Fig. 4) occurred at a similar time (1.5 h) in both the fed and fasted dogs and was similar to the plasma $T_{max}$ in non-lymph cannulated animals (1–1.5 h, Table II).

The cumulative lymphatic transport of CP532,623 following administration of 2 g of the SMEDDS formulation to fasted animals (26.3% of the dose) was similar to that obtained for CP524,515 following administration of 1 g of the same formulation. However, following post-prandial administration the increase in cumulative lymphatic transport of CP532,623 (to 36.5% of the dose) was significantly lower than that of CP524,515 (Table IV). The maximum rate of lymphatic transport of CP532,623 (Fig. 3)

**Table II** Single Dose Pharmacokinetics of CP524,515 After IV Administration (4 mg) as an Intralipid Emulsion and Oral Administration (50 mg) in a Single 1 g Capsule Containing a Long-Chain Lipid-Based SMEDDs Formulation to Non-lymph-Cannulated Fasted or Fed Greyhound Dogs and to Lymph-Cannulated Fasted and Fed Greyhound Dogs. Data are Presented as Mean ± SEM ($n=3$) or Median for $T_{max}$ Data.

| | IV | Oral | | | |
|---|---|---|---|---|---|
| | Non-lymph cannulated | Non-lymph cannulated | | Lymph cannulated | |
| | Fed | Fasted | Fed | Fasted | Fed |
| $AUC_{0\text{-}t}$ (ng.h.mL$^{-1}$) | 417±82 | 2,755±613 | 5,080±620[b] | 783±58[c] | 425±132[c] |
| $AUC_{0\text{-}\infty}$ (ng.h.mL$^{-1}$) | 502±104 | 2,861±620 | 5,230±670[b] | 1,010±94[c] | 565±99[a,c] |
| $C_{max}$ (ng/mL) | | 516±123 | 2,030±428[b] | 150±27[c] | 114±68[c] |
| $T_{max}$ (h) | | 1.5 (1.5–2.0) | 1.0 (1.0–1.0) | 2.5 (1.5–4) | 1.5 (1.5–2.5) |
| $T_{1/2}$ (h) | 3.9±0.8 | 5.4±0.4 | 5.3±1.0 | 4.3±1.2 | n.d. |
| Cl (mL.kg$^{-1}$h$^{-1}$) | 284±57 | | | | |
| $V_{Darea}$ (mL/kg) | 1,514±201 | | | | |

n.d. could not be determined as insufficient data above the limit of quantitation was available to accurately define the terminal elimination phase

[a] calculated using terminal elimination half-life for the IV-dosed animals

[b] significantly greater than that in fasted, non-lymph-cannulated dogs ($\alpha<0.05$)

[c] significantly less than that in fasted and fed, non-lymph-cannulated dogs ($\alpha<0.05$)

occurred over a similar timescale to CP524,515 (1–1.5 h) and was also consistent with the plasma $T_{max}$ values observed in the fed and fasted non-lymph cannulated dogs (Table III).

Coincident with the positive effect of food on the lymphatic transport of CP524,515 and CP532,623, there was a substantial increase in the cumulative recovery of TG in lymph in fed when compared to fasted dogs (Fig. 5). In the groups administered CP524,515, there was a significant ($\alpha<0.05$) increase (from 2.2±0.2 to 22.3±1.1 g) in cumulative TG transport in the lymph over 10 h. Similarly, TG transport in lymph increased significantly ($\alpha<0.05$, from 3.4±0.9 g to 18.4±1.4 g over 10 h) in the fed compared to the fasted groups administered CP532,623. The fasted state TG transport was greater (although not significantly) in the dogs administered CP532,623 rather than CP524,515, likely reflecting the slightly higher (2 g *versus* 1 g) quantity of long chain SMEDDS given to these animals. Surprisingly, given that the quantity of food administered to both cohorts of animals was the same, fed state lymphatic TG transport was significantly ($\alpha<0.05$) lower in the animals administered CP532,623 and this was

**Table III** Single Dose Pharmacokinetics of CP532,623 After IV Administration (4 mg) as an Intralipid Emulsion and Oral Administration (50 mg) in Two 1 g Capsules Containing a Long-Chain Lipid-Based SMEDDs Formulation to Non-lymph-Cannulated Fasted or Fed Greyhound Dogs and to Lymph-Cannulated Fasted and Fed Greyhound Dogs. Data are Presented as Mean ± SEM ($n=3$, $n=2$ for Fed Non-lymph-Cannulated Dogs) or Median for $T_{max}$ Data.

| | IV | Oral | | | |
|---|---|---|---|---|---|
| | Non-lymph cannulated | Non-lymph cannulated | | Lymph cannulated | |
| | Fed | Fasted | Fed ($n=2$)[c] | Fasted | Fed |
| $AUC_{0\text{-}t}$ (ng.h.mL$^{-1}$) | 242±7 | 1,252±223 | 1,743±229[a] | 344±51[b] | 211±59[b] |
| $AUC_{0\text{-}\infty}$ (ng.h.mL$^{-1}$) | 252±14 | 1,386±210 | 1,821±241[a] | 383±60[b] | 235±64[b] |
| $C_{max}$ (ng/mL) | | 331±48 | 796±145[a] | 130±27 | 60±15[b] |
| $T_{max}$ (h) | | 2.0 (1.5–2.0) | 1.0 (1.0–1.0) | 1.5 (1.5–1.5) | 1.5 (1.0–2.0) |
| $T_{1/2}$ (h) | 2.0±0.5 | 11.9±2.6 | 8.5±0.5 | 3.8±0.3 | 3.0±0.4 |
| Cl (mL.kg$^{-1}$h$^{-1}$) | 636±17 | | | | |
| $V_{Darea}$ (mL/kg) | 1,877±482 | | | | |

[a] significantly greater than that in fasted, non-lymph-cannulated dogs ($\alpha<0.05$)

[b] significantly less than that in fasted and fed, non-lymph-cannulated dogs ($\alpha<0.05$)

[c] data obtained only for $n=2$ animals since third animal ate only a portion of the administered food

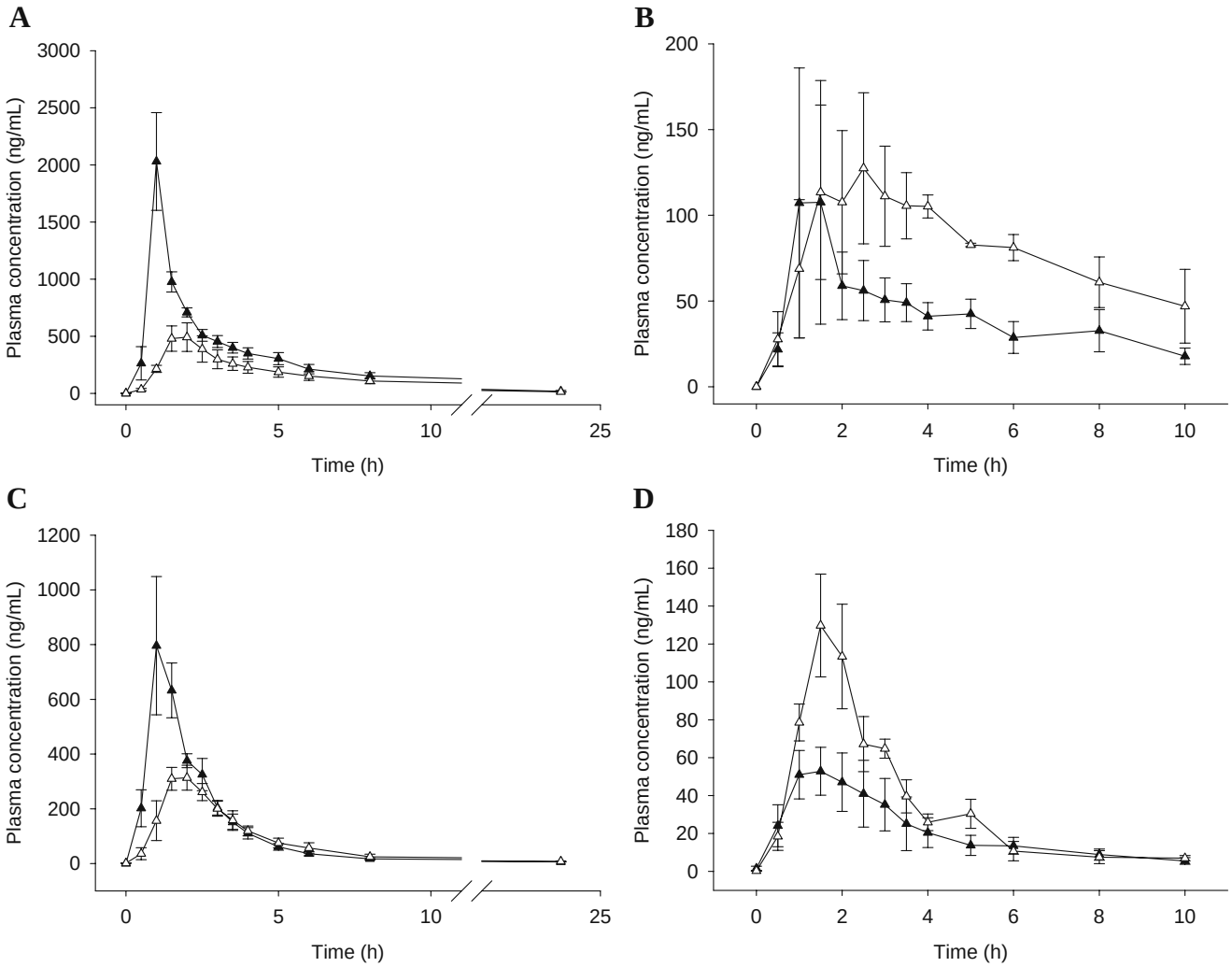



**Fig. 2** Systemic plasma concentration-time profiles of CP524,515 after oral administration with one 1 g capsule of long-chain lipid-based SMEDDS formulation to **A.** non-lymph-cannulated and **B.** lymph-cannulated dogs and of CP532,623 after oral administration with two 1 g capsules of long-chain lipid-based SMEDDs formulation to **C.** non-lymph-cannulated and **D.** lymph-cannulated dogs. Profiles show comparison of data obtained after oral administration to fasted (Δ) or fed (▲) dogs. Data presented as mean ± SEM ($n = 3$ for all groups except the fed non-lymph-cannulated group administered CP532,623 in profile C where $n = 2$ as the third animal ate only a portion of the administered food).

particularly noticeable at early time points where lymphatic TG transport was up to 50% lower (Fig. 6). The maximum reduction in lymphatic TG transport occurred coincidently with the period of maximum CP532,623 concentrations in lymph and plasma.

Fig. 2B and D show the plasma concentration-time profiles obtained following administration of CP524,515 and CP532,623 to fasted and fed lymph-cannulated dogs. These data represent the plasma exposure resulting from absorption of CP524,515 or CP532,623 directly into the blood, rather than the lymph (since the entire volume of lymph is collected in lymph-cannulated dogs). As expected, the plasma levels in lymph-cannulated animals were lower than the equivalent profiles in non-lymph cannulated animals reflecting the significant proportion of the dose that was transported into the systemic circulation via the lymph. Interestingly, and in contrast to the profiles obtained in non-lymph cannulated animals, the plasma concentration-time profiles were relatively flat and plasma AUC and $C_{max}$ decreased in fed when compared to fasted lymph-cannulated dogs (Tables III and IV). After post-prandial administration, therefore, the lymphatic transport of both CP524,515 and CP532,623 increased whilst the corresponding plasma exposure resulting from absorption directly into the blood decreased.

## Contribution of Lymph and Portal Vein Absorption to Total Bioavailability and Comparison Between Lymph-Cannulated and Non-lymph-Cannulated Dogs

Table IV provides a comparison of the total oral bioavailability ($F_{total}$) in lymph-cannulated and non-lymph

**Table IV** The Systemic Availability of a 50 mg Oral Dose of A. CP524,515 and B. CP532,623 Administered with One or Two 1 g Capsules of Long-Chain Lipid-Based SMEDDs Formulation, Respectively, to Fed or Fasted Greyhound Dogs. Data are Expressed as Percentages of the Administered Dose Absorbed Via the Lymphatic System ($F_{lymph}$) or Portal Vein ($F_{blood}$) in Lymph-Cannulated Dogs, and as the Absolute Bioavailability ($F_{total}$) in Both Lymph-Cannulated and Non-lymph-Cannulated Dogs. The Proportion (as a %) of the Systemic Availability Arising from Lymphatic Transport is Also Given. Data are Presented as Mean ± SEM ($n=3$ Dogs or $n=2$ Dogs Where Specified).

| A. CP524,515 | Fasted | | Fed | | B. CP532,623 | Fasted | | Fed | |
|---|---|---|---|---|---|---|---|---|---|
| | Lymph cannulated | Non-lymph cannulated | Lymph cannulated | Non-lymph cannulated | | Lymph cannulated | Non-lymph cannulated | Lymph cannulated | Non-lymph cannulated ($n=2$)[a] |
| $F_{lymph}$ | 25±0.8 | n.d. | 56±2.1 | n.d. | $F_{lymph}$ | 26±3.8 | n.d. | 36±1.3 | n.d. |
| $F_{blood}$ | 16±1.1 | n.d. | 9±1.3 | n.d. | $F_{blood}$ | 12±1.6 | n.d. | 7.5±1.7 | n.d. |
| $F_{total}$ | 41±0.8 | 45±8.1 | 65±1.5 | 83±8.7 | $F_{total}$ | 38±2.2 | 44±5.5 | 43±0.6 | 58±7.6 |
| Proportion via lymph | 61±2.3 | n.d. | 86±2.1 | n.d. | Proportion via lymph | 68±5.6 | n.d. | 83±3.6 | n.d. |

[a] data obtained only for $n=2$ animals since the third animal ate only a portion of the administered food

cannulated dogs and the proportion of the bioavailable fraction which was absorbed via the lymphatic system in lymph-cannulated dogs following administration in both the fed and fasted states. In all cases, intestinal lymphatic transport was the major (>60%) route of drug transport to the systemic circulation. The proportion of the total bioavailable dose that was derived from drug transport to the systemic circulation via the intestinal lymph was similar for both CP524,515 and CP532,623 and increased from approximately 65% (61±2.3% and 68±5.6%, respectively) after fasted administration to approximately 85% (86±2.1% and 83±3.6%) after post-prandial administration. The increase in overall bioavailability in the fed state appeared to be mediated entirely by increases in lymphatic drug transport since drug absorption directly into the blood ($F_{blood}$) actually decreased in fed *versus* fasted animals. Good agreement in $F_{total}$ in lymph-cannulated and non-lymph cannulated animals was apparent after fasted administration of both CP532,623 and CP524,515. After post-prandial administration, however, $F_{total}$ was slightly lower (~25%) in lymph-cannulated *versus* non-lymph cannulated animals potentially indicating a reduced capacity for the animals which had undergone surgery to process and digest food as efficiently. Nonetheless, the decrease in total absorption was similar for both drugs and as such comparison of the relative importance of lymph *versus* blood using the cannulated data for both drugs seems justified.

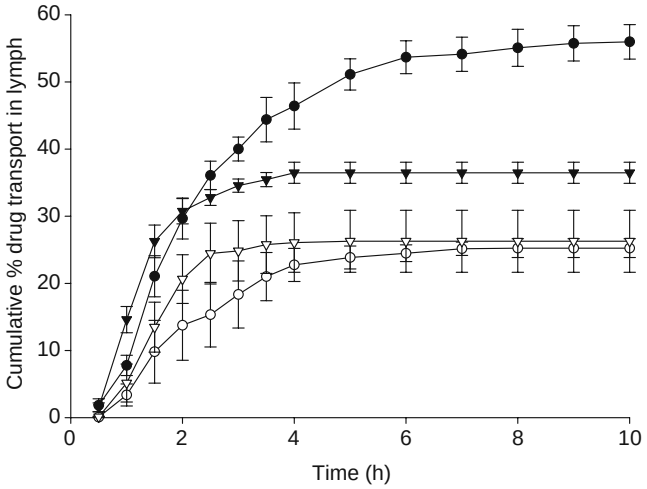



**Fig. 3** Cumulative lymphatic transport of drug (% of dose) in thoracic lymph-cannulated dogs (Mean ± SEM, $n=3$) following oral administration of 50 mg CP524,515 in a single 1 g capsule containing a long-chain lipid-based SMEDDs formulation to fasted dogs (○) or fed dogs (●) and following oral administration of 50 mg CP532,623 in two 1 g capsules of a long-chain lipid based SMEDDs formulation to fasted dogs (▽) or fed dogs (▼).

## DISCUSSION

The intestinal lymphatic system is increasingly being recognised as a potentially important route of transport to the systemic circulation for highly lipophilic drugs following oral administration (15). The prevalence of drug candidates with physicochemical characteristics consistent with significant lymphatic transport (ie high lipophilicity and low water solubility) is also increasing. The more frequent identification and development of highly lipophilic drug candidates appears to reflect both increasing utilisation of high throughput screening technologies in drug discovery (where low aqueous solubility may not limit *in vitro* activity) and the downstream application of drug delivery technologies (eg lipid based formulations, solid dispersions, nanocrystal technologies) that better support the effective absorption of poorly water soluble molecules (30).

The current study describes the effect of food on the oral bioavailability and lymphatic transport of two novel CETP

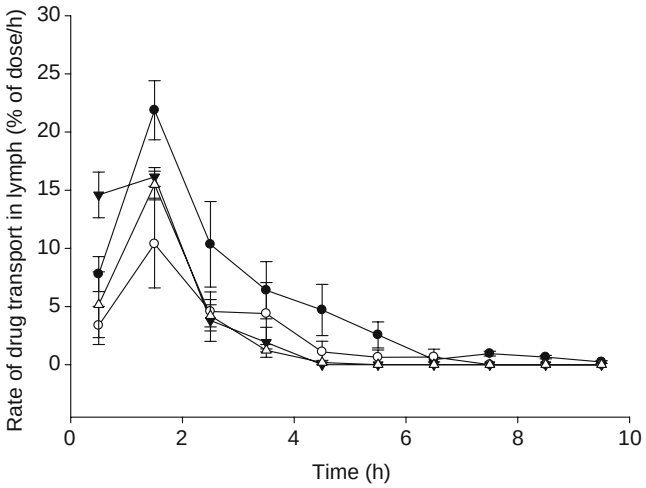



**Fig. 4** The rate of lymphatic drug transport (% of dose/h) in thoracic lymph-cannulated dogs (Mean ± SEM, $n=3$) following oral administration of 50 mg CP524,515 in a single 1 g capsule containing a long-chain lipid-based SMEDDs formulation to fasted dogs (○) or fed dogs (●) and following oral administration of 50 mg CP532,623 in two 1 g capsules of long-chain lipid-based SMEDDs formulation to fasted dogs (Δ) or fed dogs (▼).

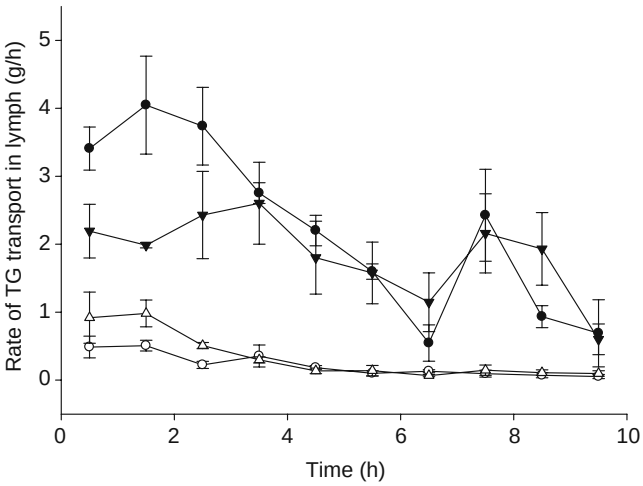



**Fig. 6** Rate of triglyceride transport in lymph (g/h) *versus* time in thoracic lymph-cannulated dogs (Mean ± SEM, $n=3$) following oral administration of 50 mg CP524,515 in a single 1 g capsule containing a long-chain lipid-based SMEDDs formulation to fasted dogs (○) or fed dogs (●) and following oral administration of 50 mg CP532,623 in two 1 g capsules of a long-chain lipid-based SMEDDs formulation to fasted dogs (Δ) or fed dogs (▼).

inhibitors, CP532623 and CP524,515. The role of the lymphatic system in the absorption profile of the CETP inhibitors was of particular interest since differences in the degree to which drug delivery to the systemic circulation occurs in association with lymph lipoproteins might be expected to impact on delivery to the site of action (ie inhibition of cholesterol ester and TG transfer between plasma lipoproteins).

The oral bioavailability of CP524,515 and CP532,623 increased 1.8 and 1.3 fold, respectively following post-prandial administration when compared to administration in the fasted state (Table IV). The positive food effect is consistent with several studies which have described increases in oral bioavailability for poorly water soluble, lipophilic drugs after co-administration with food. Food is thought to enhance the absorption of lipophilic drugs via mechanisms including slowed gastric emptying (and therefore increased time for dissolution), improved drug solubilisation within the small intestinal milieu and recruitment of intestinal lymphatic drug transport (11). In the current studies, $T_{max}$ occurred earlier in fed when compared to fasted dogs and therefore absorption appears to have occurred more rapidly post-prandially. The speed of absorption post-prandially ($T_{max} \sim 1$ h) therefore suggests that any changes in gastric empting were not large and that increases in bioavailability likely reflected changes to solubilisation and/or lymphatic transport.

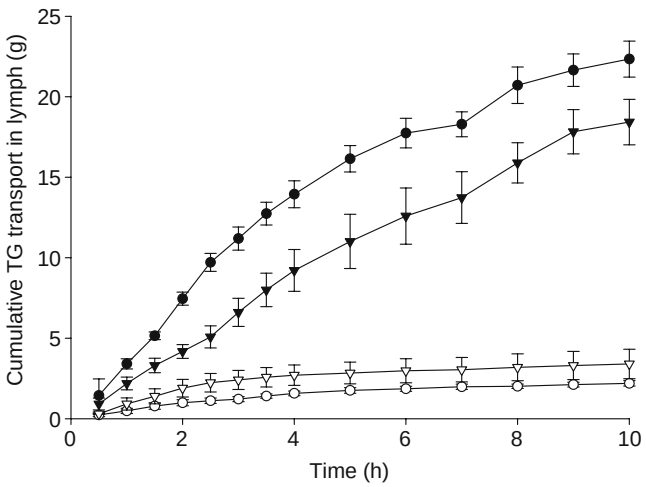



**Fig. 5** Cumulative lymphatic transport of triglyceride (g) in thoracic lymph-cannulated dogs (Mean ± SEM, $n=3$) following oral administration of 50 mg CP524,515 in a single 1 g capsule containing a long-chain lipid-based SMEDDs formulation to fasted dogs (○) or fed dogs (●) and following oral administration of 50 mg CP532,623 in two 1 g capsules of a long-chain lipid-based SMEDDs formulation to fasted dogs (▽) or fed dogs (▼).

Interestingly, the oral bioavailability of CP532,623 and CP524,515 was essentially the same after fasted administration (44.0% *vs* 45.6%, respectively) and the differences in food effect appeared to be evident only in lower post-prandial bioavailability of CP532,623 when compared to CP524,515 (57.8% *vs* 83.4%). The relatively robust fasted oral bioavailability of these highly lipophilic, poorly water soluble compounds likely reflects formulation in a long chain lipid based SMEDDS formulation. Lipid-based formulations enhance the oral bioavailability of poorly water soluble drugs by several potential mechanisms including the provision of a reservoir of dispersed lipidic microenvironments in the GI tract which maintain drug solubilisation and prevent drug precipitation (11,12). Recent studies further suggest that a key aspect of the design of lipid based formulations is appreciation of the

likely properties of the solubilised species that are formed in the GI tract after the digestion of lipidic formulation components and interaction of these digestion products with endogenous biliary derived materials including bile salt and phospholipid micelles (11,12). Lipid based formulations also support intestinal lymphatic transport, albeit typically less effectively than co-administration with the larger quantities of lipid present in food (16,17). The formulation employed in the current studies is similar to that employed in the commercial cyclosporine formulation (Neoral®) and essentially identical to formulations that have previously been shown to enhance the oral bioavailability of similarly lipophilic drugs such as halofantrine and danazol when compared with, for example, a simple solid dose formulation (31,32).

The larger increase in post-prandial bioavailability of CP524,515 when compared with CP532,623 may reflect its higher lipid solubility and therefore potentially enhanced affinity for dispersed lipidic microdomains in the intestine such as mixed micelles, vesicles and emulsion droplets. More detailed examination of the differences in oral bioavailability between CP532,623 and CP524,623, however, are complicated by the fact that the lower lipid solubility of CP532,623 dictated administration in association with 2 g of the long chain lipid SMEDDS formulation rather than 1 g in the case of CP524,515. This approach was taken to facilitate comparison of absorption profiles after administration of the same drug dose and in the same formulation, but leaves open the possibility that the fasted state bioavailability of CP532,623 may have been higher than that of CP524,515 by virtue of co-administration with a larger quantity of formulation. Under these circumstances it is possible that the increase in oral bioavailability after co-administration with food for CP532,623 was attenuated by the relatively higher solubilising power of the larger quantity of formulation administered in the fasted state when compared with CP524,515. This is consistent with previous studies that have shown that lipid based formulations such as those utilised here may reduce or ameliorate the effect of food on the oral bioavailability of poorly water soluble, lipophilic drugs (17,31,32). Subsequent studies will address the dependence of the bioavailability of the CETP inhibitors on the quantity of formulation administered.

After oral administration of 1 g of a long-chain SMEDDS formulation containing 50 mg (~1.6 mg/kg) of CP524,515, the majority of the drug dose was transported to the systemic circulation via the intestinal lymph, even after fasted administration (25% of the dose over 10 h) (Table IV). The extent of lymphatic transport increased further after co-administration with food (56% of the dose over 10 h) in parallel with a post-prandial increase in lymphatic TG transport from 2.2 to 22 g over 10 h. These data are highly consistent with previous studies which explored the lymphatic transport of a similarly lipophilic antimalarial, halofantrine (clog P 8.5 and TG solubility >50 mg/g in soybean oil) where 28% of a 1.6 mg/kg dose was lymphatically transported following administration with 1 g of essentially the same long-chain SMEDDS formulation in the fasted state and 54% of a 3.2 mg/kg dose was recovered in lymph following post-prandial administration (in the latter case with a lipid-free formulation) (16,17).

In contrast to CP524,515, CP532,623 was not expected to be substantially absorbed via the intestinal lymphatic system since its solubility in long-chain TG (Table I) was considerably lower than that typically seen for lymphatic transport candidates (19). Thus, compounds including seocalcitol (33), saquinavir (34), hexachlorobenzene (19) and PRS-211,220 (35), all have similar physicochemical properties to CP532,623 (LogD >5 but long chain TG solubility <50 mg/g) and are poorly transported via the lymphatic system following oral delivery. Surprisingly, however, the extent of lymphatic transport of CP532,623 after fasted administration was comparable to that of CP524,515 and halofantrine, albeit after administration with a larger quantity (2 g) of the long-chain SMEDDS formulation (leading to an increase in lymphatic TG transport over 10 h after fasted administration from 2.2± 0.2 to 3.4±0.9 g for animals administered 1 g and 2 g of formulation lipid respectively). We believe this is the first study to report substantial (>20% of the dose) lymphatic transport of a drug (CP532,623) with long-chain TG solubility <50 mg/g.

The current data therefore indicate that literal application of historical guidelines which suggest that lymphatic transport is only likely for compounds with LogP's in excess of 5 and long chain TG solubilities of >50 mg/g should be approached with caution. The previous guidelines were based (in part) on a comparison of the extents of lymphatic transport of DDT (high LogP (6.19), high lipid solubility (97.5 mg/g in peanut oil)) and hexachlorobenzene (high LogP (6.53) but lower lipid solubility (7.5 mg/g in peanut oil)), where significant lymphatic transport was only evident for DDT (19). The suggestion that high lipid solubility (in addition to a high partition co-efficient) was required was based on the assumption that most drugs were transported into the lymph within the TG rich core of lipoproteins (19,36). Recent studies by Gershkovich *et al.*, however, have shown that drug solubility in an artificial TG emulsion (Intralipid®) may not be highly predictive of drug association with TG rich lipoproteins (and subsequent transport into the lymph) and therefore that for some drugs lipoprotein association may be a complex process dictated by drug association with the TG rich core and/or interaction with surface components of lipoproteins, including apo-proteins (21,22). A possible explanation, therefore, for the high extent of lymphatic

transport of CP532,623 in the current studies despite a modest TG solubility may be that other components of TG rich lipoproteins, besides TG, facilitate lipoprotein association of CP532,623 and therefore transport into the lymph. Further studies are ongoing to more specifically probe the mechanism(s) underlying the extensive lymphatic transport of CP532,623.

The increase in lymphatic transport on administration of CP532,623 with food (from 26 to 36%) (Table IV) was not as great as that seen for CP524,515 (from 25 to 56% of the administered dose) or previously for halofantrine (from 28 to 54% of the administered dose) (16,17). It seems likely, however, that this reflects a smaller increase in fed-state absorption since the post-prandial bioavailability of CP532,623 was significantly lower than CP524,515 (Table IV), and the proportion of the available drug dose which was absorbed via the lymphatic system was similar for CP532,623 and CP524,515 in both the fed and fasted state (Table IV).

The majority of the bioavailable fractions of both CP524,515 and CP532,623 were therefore transported into the systemic circulation via the lymphatic system following fasted (61 and 68%) and post-prandial (86 and 83%) administration (Table IV). Consistent with the critical role of the lymphatics in delivering drug to the systemic circulation, the maximum plasma concentrations for both drugs (Table II and III) also occurred at the approximate time of maximum lymphatic drug flux (Fig. 4). As a result of the primary role for intestinal lymphatic transport in drug absorption, a much smaller proportion of the bioavailable drug fraction was absorbed via the blood after fasted administration (38.9% and 31.6% for CP524,515 and CP532,623 respectively). Further, the quantity of drug absorbed into the blood after post prandial administration actually decreased (Fig. 2), in spite of the overall increase in bioavailability. Similar trends have been reported previously for halofantrine (16,17) and likely reflect increased drug partitioning into lymph lipoproteins post-prandially due to expansion of the lymphatic lipid pool (37), enhanced synthesis of lymph lipoproteins and increased lymphatic lipid transport.

Finally, the lymph TG transport data in the current study suggests that compounds in the torcetrapib series of CETP inhibitors may influence intestinal lipoprotein processing, at least under relatively high lipid loads. Cumulative lymphatic TG transport (Fig. 5) and the rate of lymphatic TG transport (Fig. 6) in the fed state was significantly lower following administration of CP532,623 when compared to CP524,515. This was particularly evident in fed animals over the 1–3 h post-dose period when systemic and lymphatic drug concentrations of CP532,623 were highest (Fig. 5, Fig. 2C). In contrast, differences between lymphatic lipid transport rates were not evident when systemic drug concentrations dropped below approximately 200 ng/mL. These effects are consistent with those seen following oral administration of the structurally related CETP inhibitor, torcetrapib, in combination with atorvastatin (when compared to no treatment or treatment with atorvastatin alone) to humans. Administration of torcetrapib with atorvastatin significantly attenuated the post-prandial increase in plasma CM and VLDL in humans with type IIB hyperlipidemia. CETP deficient humans also display a diminished or delayed post-prandial increase in plasma TG (38–40).

The principle mode of action of the CETP inhibitors is thought to be inhibition of lipid transfer between high density lipoprotein and lower density lipoproteins within the systemic circulation (1,2,6). The current studies raise the interesting possibility that at least some CETP inhibitors (eg. CP532,623) may affect lipoprotein assembly and lipid transport through the enterocyte into the lymph. Whilst dogs do not express functional CETP in plasma (41,42), CETP and an alternate isoform of CETP (CETPI) have been identified in the small intestine of humans (43) and rabbits (44), raising the possibility that the effects of CP532,623 on intestinal lipoprotein processing may be mediated via inhibition of CETP in the intestine of dogs (the expression of CETP in the intestine of dogs appears not to have been studied). Alternatively, the CETP inhibitors may interact with targets such as microsomal TG transfer protein (MTP) (45–47) or phospholipid transfer protein (PLTP) (48–50), which are both known to influence intestinal lipoprotein processing and share significant structural homology with CETP (51,52). The effect of CP532,623 on intestinal lipid transport warrants further investigation as effects on lipid and lipoprotein processing within tissues, in addition to the well known effects of the CETP inhibitors in plasma, may contribute to the activity and toxicity profiles of CETP inhibitors in the treatment of atherosclerosis.

## CONCLUSION

Intestinal lymphatic transport is a significant contributor to the oral bioavailability of CP524,515 and CP532,623 following oral administration to both fasted and fed thoracic lymph duct-cannulated greyhound dogs. Food enhanced the oral bioavailability of both compounds and also increased the proportion of the absorbed dose that was transported to the systemic circulation via the lymph (from ~65% after fasted administration to ~85% post-prandially). The physicochemical properties of CP524,515 were broadly consistent with previous examples of highly lymphatically transported drugs, whereas the extent of lymphatic transport of CP532,623 was surprisingly high bearing in mind its limited

solubility in long chain triglyceride (<20 mg/g). The mechanism of lymphatic access of CP532,623 is currently under investigation. Finally, CP532,623, reduced post-prandial lymphatic triglyceride transport by up to 50% (when compared to CP524,515) over timescales where systemic drug levels were highest (0–2 h post dose), raising the possibility of a previously undefined pharmacological activity.

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