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Research Paper

Intestinal Lymphatic Transport Enhances the Post-Prandial Oral Bioavailability of a Novel Cannabinoid Receptor Agonist Via Avoidance of First-Pass Metabolism

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Purpose. To examine the effect of food on the oral bioavailability of a highly lipophilic, cannabinoid receptor agonist (CRA13) and to explore the basis for the food effect in lymph-cannulated and non-cannulated dogs.
Methods. Oral bioavailability was assessed in fasted and fed human volunteers and in lymph-cannulated dogs. In fasted dogs, the extent of absorption and oral bioavailability was also examined following administration of radiolabelled CRA13.
Results. Food had a substantial positive effect on the oral bioavailability of CRA13 in human volunteers (4.3–4.9 fold increase in $AUC_{0-\infty}$) and in dogs. The absolute bioavailability of parent drug was low in fasted dogs (8–20%), in spite of good absorption (72–75% of radiolabelled CRA13 recovered in the systemic circulation). In post-prandial lymph-cannulated dogs, bioavailability increased to 47.5% and the majority (43.7%) of the dose was absorbed via the intestinal lymphatic system.
Conclusions. The positive food effect for CRA13 does not appear to result from increased post-prandial absorption. Rather these data provide one of the first examples of a significant increase in bioavailability for a highly lipophilic drug, which is stimulated via almost complete post-prandial transport into the lymph, in turn resulting in a reduction in first-pass metabolism.

KEY WORDS: cannabinoid; first-pass metabolism; food effect; lymphatic transport; oral bioavailability.

INTRODUCTION

The most well recognised and common pathway of access to the systemic circulation following oral drug delivery is via uptake into enterocytes (intestinal absorptive cells) and transport from the intestine to the systemic circulation via the portal vein. It is becoming increasingly apparent, however, that transport into the systemic circulation via the intestinal lymphatic system may constitute an alternate, and potentially important, contributor to systemic access following oral delivery of some highly lipophilic drugs (1–5). Drug transport to the systemic circulation in association with intestinal lymph has the potential to alter drug distribution and systemic clearance (1,2,6,7) and may also impact on the extent of first-pass metabolism and oral bioavailability. Promotion of intestinal lymphatic transport has been shown to reduce first-pass metabolism within the enterocyte (by altering intracellular drug distribution patterns) (8) and by the liver (since, unlike the portal blood, the intestinal lymph empties directly into the systemic circulation without first passing through the liver) (9,10).

Drug access to the intestinal lymph occurs via association with lymph lipoproteins [primarily chylomicrons (CM) and very low density lipoproteins (VLDL)] (11,12) that are assembled in the enterocyte in response to the absorption of dietary or formulation-derived lipids (13,14). As such, common features of drugs that are transported via the intestinal lymph are high lipophilicity (typically log $P>5$ and long-chain triglyceride solubility >50 mg/g) (1,15,16) and significant increases in bioavailability after administration with the large quantities of lipid contained in food (10). However, increases in post prandial bioavailability are common for many highly lipophilic, poorly water soluble drugs since food typically enhances both lymphatic drug transport and drug solubilisation in the lumen of the gastrointestinal tract (1). The

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ABBREVIATIONS: CM, Chylomicron; CRA, Cannabinoid receptor agonist; HDL, High density lipoprotein; LDL, Low density lipoprotein; TG, Triglyceride; VLDL, Very low density lipoprotein.

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mechanisms underpinning increases in post-prandial bioavailability (i.e. increased luminal solubilisation or reduced first pass metabolism via increased lymphatic transport) therefore cannot be deconvoluted from a simple food effect study.

An understanding of the potential role of intestinal lymphatic transport in oral drug bioavailability is critical since the extent of lymphatic transport is highly formulation- and food-dependent and therefore has implications in both formulation design and labelling requirements. Whilst lymphatic transport has been demonstrated to be a significant absorption pathway for only relatively few marketed drugs (1), empirical evidence suggests that the number of drug development candidates with potential lymphatic involvement is increasing (due in large part to the well acknowledged trend toward the discovery of more lipophilic drug candidates) (17). An early understanding of the potential involvement of lymphatic transport in oral bioavailability during drug development is therefore of increasing importance, given that alterations to lymphatic transport may impact on exposure and that the extent of lymphatic transport is highly formulation dependent (18).

CRA13 (Fig. 1, also compound 13 in (19)) is a cannabinoid receptor agonist (20,21) currently under investigation for the management of chronic pain and is a typical example of a highly lipophilic development candidate for which food is likely to have a substantial positive effect on absorption. The physicochemical profile and affinity of CRA13 for lipoproteins also suggests the potential involvement of lymphatic transport in drug absorption following oral delivery. CRA13 has a log *P* of 6.9, sesame oil solubility of 80 mg/ml and aqueous solubility of 19 μg/ml. In human plasma the total protein binding of CRA13 is high (>99.5%) with extensive distribution into plasma lipoprotein fractions (approximately 30% of protein-bound CRA13 is associated with VLDLs, 30% with LDLs and 20% with HDLs, as such approximately 80% of the overall plasma protein binding profile of CRA13 is due to association with plasma lipoproteins; data on file, Novartis AG, Switzerland).

In this communication we report a series of studies that have been undertaken to more closely probe the effect of food on oral bioavailability, and the role of lymphatic transport in the post prandial absorption profile of CRA13. First, a human study is presented which examines the effect of food on the bioavailability of CRA13 after oral administration. Second, a bioavailability study in fasted beagle dogs is presented using radiolabelled CRA13 to demonstrate that CRA13 is well absorbed after oral administration, and therefore that the enhancement in post prandial bioavailability likely reflects changes to first pass metabolism, rather than absorption. Finally we report the use of a greyhound dog model to directly examine the role of intestinal lymphatic transport of CRA13 in post-prandial oral bioavailability.

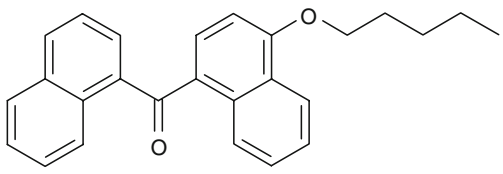

Fig. 1. Structure of CRA13.

Unfortunately, direct evaluation of lymphatic drug transport in humans is not possible due to the non-reversible nature of the invasive surgery required to access and cannulate the intestinal and/or thoracic lymphatic duct. However, the greyhound dog model is well described in the literature (10,22,23) and allows examination of the lymphatic transport of CRA13 after oral administration of the same formulation that was employed in the clinical study. The greyhound model also allows administration under representative post-prandial (fed) conditions in an animal model where the gastrointestinal tract, gastric transit profile and biliary secretion patterns are more similar to humans than, for example, small animal models such as the rat (24,25).

MATERIALS AND METHODS

Materials

Tritiated CRA13 ([^{3}H]-CRA13, 2-[^{3}H]-naphthalen-1-yl)-(2-[^{3}H]-4-pentyloxy-naphthalen-1-yl)-methanone, batch 0079-92) was supplied by the isotope laboratory of Novartis Pharma East Hanover (USA). Deuterated CRA13 ([D_{11}]-CRA13, batch N0186-20) and unlabelled CRA13 (batch KIL6242/03.7) were supplied by Novartis Pharma AG (Basel, Switzerland). All other chemicals were of analytical reagent grade and solvents were of HPLC grade.

Oral Capsule and Liquid Formulations of CRA13 for Administration to Humans

Human volunteers were administered a capsule formulation containing 150 mg of a solidified micellar solution of CRA13 comprising 15 mg of CRA13, 67.5 mg Cremophor RH40 and 67.5 mg polyethyleneglycol 4000 which was filled in the liquid state into hard gelatin capsules and cooled to room temperature prior to administration.

Intravenous and Oral Formulations of [^{3}H]-CRA13/CRA13 for Administration to Beagle Dogs

For intravenous administration to beagle dogs, a 2 mg/mL solution of CRA13 (including 0.162 μCi/mL [^{3}H]-CRA13) in ethanol/PEG200/glucose 5% (*w*/*v*) (1:7:2 *v*/*v*/*v*) was utilised. The test compound ([^{3}H]-CRA13 plus unlabelled compound) was first dissolved in ethanol before the addition of PEG200 followed by glucose 5% (*w*/*v*). The formulation was kept under magnetic agitation throughout the preparation procedure and was occasionally subjected to ultrasonication. The solution was heated (30°C) for 10–15 min to give a clear dosing solution.

For oral administration to beagle dogs, a micellar solution comprising Cremophor RH40/propylene glycol/water (8:2:190, *w*/*w*/*w*) and containing 0.1 mg/mL CRA13 (including 9.96 nCi/mL [^{3}H]-CRA13) was employed. The test compound ([^{3}H]-CRA13 plus unlabelled compound) was first dissolved in propylene glycol and Cremophor RH40 before the addition of water. The formulation was kept under magnetic agitation throughout the preparation procedure and was occasionally subjected to ultrasonication. The formulation appeared as a slightly opalescent solution.

Oral Capsule Formulation of CRA13 for Administration to Lymph-Duct Cannulated Greyhounds

An identical formulation to that employed in the clinical study was employed in the greyhound studies. Lymph duct-cannulated greyhound dogs were administered two capsules, each filled with 15 mg CRA13 formulated as a solidified micellar solution. The mean weight of the animals in the study was 31 kg. This provided a dose that was as close to the 1 mg/kg administered to the beagle dogs as possible when administering a unit dose capsule.

Experimental and Surgical Procedures

1. Oral pharmacokinetic and bioavailability studies in humans

Ethics approval for the human studies was obtained from the Covance Clinical Research Unit Independent Review Board, Leeds, UK. The study was a randomized, open-label, three period, single oral dose crossover design study, with a total of 12 male human subjects chosen for the study. All volunteers provided written informed consent for the study. Criteria for inclusion were that the subjects were healthy, non smoking, male volunteers aged between 18 and 45 years. The intake of methylxanthine (e.g. caffeine) containing food or beverages, alcohol, and strenuous physical exercises were restricted during study participation. Each subject received one capsule containing 15 mg CRA13 as the solidified micellar formulation filled into hard gelatin capsules. Formulations were administered after an overnight fast (10 h), with a washout period of at least 2 weeks between the two administrations. All volunteers continued to fast for an additional 4 hours post-dose. After a further 2-week washout period, six of the 12 subjects received one capsule containing 15 mg CRA13 in the solidified micellar formulation 10 min after a standard FDA-recommended high fat breakfast with 40% fat content (26). Blood samples for the determination of CRA13 concentrations in plasma (2 mL) were collected by either direct venipuncture or via an indwelling cannula and placed into lithium heparin tubes. Blood samples were drawn from a forearm vein within 30 min before dosing and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96 h after drug administration. The samples were centrifuged at 3–5°C for 15 min at approximately 1,500×*g*. The plasma was transferred into polypropylene screw-cap tubes and samples were stored frozen at ≤−18°C, pending analysis.

2. Pharmacokinetic and bioavailability studies in beagle dogs

All surgical and experimental procedures were approved by the local Institutional Animal Experimentation Ethics Committee. Three healthy male beagle dogs (10–11.5 kg) were given CRA13 via the intravenous route and one dog (11.2 kg) was sacrificed 7 days after intravenous administration of CRA13 to determine the whole body distribution of remaining drug and metabolites. The two remaining dogs were given a per-oral dose of CRA13, 5 weeks later. Each dog received their last feed at noon on the day before CRA13 administration; food was given 6 h post-dose and tap water was available *ad libitum*.

IV Dosing to Beagles. The IV formulation contained 2 mg/mL (total) of [^{3}H]-CRA13 and unlabelled CRA13 and was administered by slow bolus injection into the foreleg vein in a volume of 0.5 mL/kg to achieve a CRA13 dose of 1 mg/kg. The administered volume was accurately determined by weighing the syringe before and after dosing. Samples (6 mL) of systemic blood were obtained via an indwelling cephalic vein cannula at: predose, 5 min, 15 min, 30 min, 1, 2, 4, 6, 8, 24, 48, 72, 96, 120, 144 and 168 h post-dose. Blood samples were collected into heparinized syringes and after centrifugal separation of plasma from red blood cells, plasma was transferred to polypropylene Eppendorf tubes which were then stored at −20°C pending analysis.

Oral Dosing to Beagles. The oral formulation contained 0.1 mg/mL of [^{3}H]-CRA13 and unlabelled CRA13 and was administered by gavage at a dose of 10 mL/kg to provide a CRA13 dose of 1 mg/kg. Samples (6 mL) of systemic blood were obtained via an indwelling cephalic vein cannula at: predose, 15 min, 30 min, 1, 2, 4, 6, 8, 24, 48, 72, 96, 120, 144 and 168 h post-dose. Blood samples were collected into heparinized syringes, and after centrifugal separation of plasma from red blood cells, plasma was transferred to polypropylene Eppendorf tubes which were then stored at −20°C pending analysis. Urine and faeces were also collected from the dogs up to 168 hours post-dose.

3. Lymphatic transport studies in greyhound dogs

Greyhound Thoracic Lymph Duct Surgical Cannulation. All surgical and experimental procedures were approved by the local Institutional Animal Experimentation Ethics Committee. Following induction of surgical anaesthesia, the thoracic lymph duct of healthy adult male greyhound dogs (27–30 kg) was cannulated as previously described (10). After surgery, the dogs were allowed to recover unrestrained for a period of 14–16 h with water available *ad libitum.* During this time, all animals returned to normal ambulatory movement. An intravenous catheter was also inserted into the cephalic vein prior to drug administration to enable serial sampling of systemic blood during the study period.

Oral Dosing to Thoracic Lymph Duct Cannulated Greyhounds. Each dog (n=3) was fed a standard can of commercial dog food (680 g) containing 5% crude fat (max.) approximately 30–45 min prior to the oral administration of 2×15 mg capsules of CRA13. In addition to *ad libitum* access to drinking water, 25 mL normal saline was administered via the cephalic vein cannula at hourly intervals to limit any possible dehydration due to the continuous collection of thoracic lymph.

Samples (5 mL) of systemic blood were obtained via a cephalic vein cannula at predose, 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 h post-dose. Blood samples were collected into heparinized blood collection vials. After centrifugal separation of plasma from red blood cells, 250 μL plasma was transferred to polypropylene Eppendorf tubes which were then stored at −80°C pending analysis.

Lymph was collected continuously into 50 mL polypropylene tubes containing 75 mg disodium EDTA (anticoagulant) for the 10 h post-dosing period. Lymph collected over each 1 h post-dose period was pooled and the total volume of

lymph collected per h determined gravimetrically (assuming a specific gravity of 1 g/mL). Aliquots (50 μL) of the pooled lymph samples from each hourly collection period were dispensed into Eppendorf tubes and stored at −80°C prior to analysis.

Analytical Procedures

Concentrations of Parent CRA13 in Human, Beagle and Greyhound Plasma and Greyhound Lymph

Plasma and lymph concentrations of CRA13 and the structurally-related internal standards were determined by LC-MS/MS following acetonitrile precipitation of plasma proteins.

Preparation of Human Plasma Samples for LC-MS/MS

Human plasma samples (200 μL) were mixed with 600 μL of internal standard solution (4 ng/mL 4-butoxy-naphthalen-1-yl)-naphthalen-1-yl-methanone in acetonitrile) in polypropylene Eppendorf tubes. Solutions were mixed on a vibration shaker for approximately 5 s, and then centrifuged (4,000×*g*) at 5°C for 10 min. Supernatant was transferred into a polypropylene Eppendorf tube and evaporated to dryness with an IR-Dancer device (Hett-Lab AG, Switzerland). The dry residues were reconstituted with 300 μL of methanol/ammonium acetate 0.05 M (1:1 *v*/*v*) and shaken for approximately 5 s on a vibration shaker before transfer into glass injection vials. The vials were capped and 100 μL injected onto the LC-MS/MS.

Preparation of Beagle Plasma Samples for LC-MS/MS

Plasma samples (200 μL) were mixed with 400 μL of internal standard solution (30 ng/mL 6-[2-(2,5-dimethoxy-phenyl)-ethyl]-4-methoxy-quinazoline in acetonitrile) and 100 μL of a saturated aqueous sodium chloride solution in polypropylene Eppendorf tubes. Solutions were mixed on a vibration shaker for approximately 5 s, and then centrifuged (4,000×*g*) at 5°C for 5 min. Supernatant (300 μL) was transferred into a glass autosampler vial, 300 μL of water added, vials capped and 200 μL injected onto the LC-MS/MS.

Preparation of Greyhound Plasma and Lymph Samples for LC-MS/MS

Lymph or plasma samples (100 μL) were mixed with 200 μL of deuterated internal standard solution (4.54 ng/mL [D_{11}]-CRA13 in acetonitrile) in polypropylene Eppendorf tubes. Solutions were mixed on a vibration shaker for approximately 5 s, and then centrifuged (4,000×*g*) at 5°C for 5 min. The supernatant was transferred into glass autosampler vials, vials capped and 200 μL injected onto the LC-MS/MS apparatus.

LC-MS/MS Measurement of CRA13 in Plasma and Lymph Samples

Plasma concentrations of CRA13 and the structurally-related internal standards were determined using an LC-MS/MS system consisting of an Agilent 1100 LC system equipped with an LC analytical column (Zorbax SB-C18, 30 mm× 4.6 mm, 3.5 μm particle size, running at a column temperature of 40°C) and a ThermoFinnigan TSQ 7000 mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source. The mass spectrometer was run in selected reaction monitoring (SRM) positive ion mode. The corona discharge was 4 μA, the dynode was set at 15 kV and the electron multiplier at 1,500 V. Argon was used as collision gas at a pressure of 3 mTorr. The remaining LC-MS/MS conditions used for analysis of CRA13 in human, beagle and greyhound samples varied slightly and details are given in Table I. The limits of quantitation (LOQ) were 1.77, 1.00 and 2.00 ng/mL for the human, beagle and greyhound plasma assays, respectively. At the LOQ, the assay was accurate to within 90–115% of target and precise to within ±15% CV respectively. Above the LOQ, the inter-day accuracy ranged from 90–110% and the inter-day precision was less than ±10% CV.

Measurement of Radiolabelled Parent and Metabolite Concentrations in Beagle Plasma

To establish the extent of absorption of CRA13, the total plasma concentration of radiolabelled parent and metabolites of CRA13 were quantified by measuring the concentration of total ^{3}H radioactivity in beagle plasma by liquid scintillation counting. Plasma samples of 50 mg were mixed with 2 mL of Irgasolv (Ciba Specialty Chemicals Basle, Switzerland)/isopropanol (1:1)). After overnight incubation, 0.5 mL of 2 N HCl was added, followed by 17.5 mL of OptiPhase 'HiSave'3® (Wallac, Turku, Finland) as a scintillant. Scintillation counting was performed using a TriCarb 2700 TR Liquid Scintillation System (Packard Instrument Co., Meridenct, CT Grove, USA). Concentrations of total ^{3}H were determined in weighed samples and converted from millimoles per gram to nanomoles per litre assuming a density of 1.0 g/mL for plasma.

Concentrations of Triglyceride in Greyhound Lymph

Following a 10-fold dilution of lymph samples in Milli-Q water, triglyceride (TG) concentrations were determined using a clinical chemistry analyser (Roche Cobas Mira, Switzerland) and a commercial enzyme-based colorimetric assay kit (Boehringer Mannheim, Germany) as described previously (18).

Data Analysis

Analysis of Plasma Pharmacokinetic Profiles

The maximum plasma concentration (C_{max}) of CRA13 (determined by LC-MS/MS), and the time to reach the maximum (t_{max}) were noted directly from the individual profiles. The area under the plasma concentration-time profiles from time zero to the last measured concentration (AUC_{0-tz}) was calculated by WinNonLin (V4.0.1, Pharsight, Apex, NC; human studies) and the linear trapezoidal method (dog studies). Where the apparent terminal phase of the plasma concentration-time profiles was evident, the area

Table I. Conditions Used for LC-MS/MS Analysis of CRA13 in Human, Beagle and Greyhound Samples

	Human	Beagle	Greyhound
Mobile phase			
Flow rate	1 mL/min	1 mL/min	1 mL/min
Composition	MPA[a]: 50 mM ammonium acetate MPB[b]: 100% methanol	MPA[a]: 50% methanol in 50 mM ammonium acetate MPB[b]: 100% methanol	MPA[a]: 20 mM ammonium acetate MPB[b]: 100% methanol
Gradient	From 100% MPA to 100% MPB varied linearly over 4.5 min, returned to 100% MPA over 2 min	From 50% MPA to 100% MPB varied linearly over 4.5 min, returned to 100% MPA over 2 min	100% MPA held for 1 min, varied linearly to 100% MPB over 30 s, held at 100% MPB for 3.3 min, returned to 100% MPA over 0.2 min and held for 2 min
MS/MS			
APCI carrier gas (N_2) pressure (psi)	50	40	40
Capillary temperature (°C)	225	250	250
Vaporiser temperature (°C)	500	500	550
Collision energy (eV)	−32 for CRA13 and IS	−36.5 and −34.7 for CRA13 and IS	−27.0 and −28.0 for CRA13 and IS
Ion pairs monitored			
CRA13	369.2→154.9	369.2→154.9	368.8→155.0
Internal standard	355.0→154.9	323.1→172.1	380.0→155.0

[a] Mobile Phase A
[b] Mobile Phase B

obtained by extrapolation to infinite time was added to AUC_{0-tz} to obtain the area from time zero to infinity, $AUC_{0-\infty}$. Apparent elimination half lives ($T_{1/2}$) and the elimination rate constants (k) for the terminal elimination phase were calculated using WinNonLin (V4.0.1, Pharsight, Apex, NC) and the plasma clearance (CL) and volume of distribution (V_D) of CRA13 after IV administration in the beagle studies were calculated using the following equations:

$$\mathrm{CL} = \mathrm{Dose}/\mathrm{AUC}^{0-\infty} \quad (1)$$

$$\mathrm{V_D} = \mathrm{Dose}/\left(k \times \mathrm{AUC}^{0-\infty}\right). \quad (2)$$

Absorption and Bioavailability Analysis in Beagles

Analysis of Radiolabelled Data. The extent of absorption of [^{3}H]-CRA13 plus ^{3}H-labelled metabolites following oral administration of [^{3}H]-CRA13 to fasted beagles was estimated from the dose-normalised AUC of all radioactive species in plasma following per-oral and IV administration of ^{3}H-CRA13, i.e.

$$\text{Extent of absorption } (\%) = \left(\mathrm{AUC}^{^3\mathrm{H}}_{\mathrm{PO}} \Big/ D^{^3\mathrm{H}}_{\mathrm{PO}}\right) \Big/ \left(\mathrm{AUC}^{^3\mathrm{H}}_{\mathrm{IV}} \Big/ D^{^3\mathrm{H}}_{\mathrm{IV}}\right) \times 100\% \quad (3)$$

where $D^{^3\mathrm{H}}_{\mathrm{PO}}$ and $D^{^3\mathrm{H}}_{\mathrm{IV}}$ are the total dose of radioactivity administered either via the per-oral or IV route, respectively and $AUC^{^3H}_{PO}$ and $AUC^{^3H}_{IV}$ represents the AUC values for all radioactive species in plasma following IV or per-oral administration, respectively.

Analysis of Parent CRA13. The absolute bioavailability of CRA13 following oral administration of CRA13 to fasted beagles (F_{total}) was calculated as the ratio of the dose-normalised AUC values for parent CRA13 (measured using LC-MS) following oral administration relative to that following IV administration, i.e.:

$$F_{\mathrm{Total}} = \left(\mathrm{AUC}^{\mathrm{CRA13}}_{\mathrm{PO}} \Big/ D^{\mathrm{CRA13}}_{\mathrm{PO}}\right) \Big/ \left(\mathrm{AUC}^{\mathrm{CRA13}}_{\mathrm{IV}} \Big/ D^{\mathrm{CRA13}}_{\mathrm{IV}}\right) \times 100\% \quad (4)$$

where $D^{\mathrm{CRA13}}_{\mathrm{PO}}$ and $D^{\mathrm{CRA13}}_{\mathrm{IV}}$ are the total doses of CRA13 (i.e. [^{3}H]-CRA13 plus unlabelled CRA13) administered via the oral and intravenous routes, respectively, and $\mathrm{AUC}^{\mathrm{CRA13}}_{\mathrm{PO}}$ and $\mathrm{AUC}^{\mathrm{CRA13}}_{\mathrm{IV}}$ represent the AUC for CRA13 (i.e. parent drug excluding metabolites, determined by LC-MS/MS) in plasma following per-oral and IV dosing, respectively.

Bioavailability Analysis and Lymph Versus Blood Absorption in Greyhound Dogs. The mass of analyte transported into thoracic lymph during each sampling period was calculated as the product of the analyte concentration and total volume of lymph collected during the sampling period. The fraction of the dose transported into lymph (F_{lymph}) was calculated as the ratio of cumulative mass of CRA13 transported into lymph and the oral dose. The proportion of the dose absorbed into the systemic circulation via the portal route ($F_{\mathrm{systemic\ blood}}$) following oral administration of CRA13 to lymph-cannulated greyhounds was estimated from the dose-normalised plasma AUC for CRA13 following oral administration to lymph-

cannulated greyhounds relative to the dose-normalised (mg/kg) plasma AUC for parent CRA13 following IV administration to beagles, i.e.

$$F_{\text{systemic blood}} = \left(\text{AUC}^{\text{CRA13}}_{\text{PO-LC}}/D^{\text{CRA13}}_{\text{PO-LC}}\right)/\left(\text{AUC}^{\text{CRA13}}_{\text{IV}}/D^{\text{CRA13}}_{\text{IV}}\right) \times 100\% \quad (5)$$

where $D^{\text{CRA13}}_{\text{PO-LC}}$ is the total dose (μg/kg) of CRA13 administered via the per-oral route to lymph-cannulated greyhound dogs and $\text{AUC}^{CRA13}_{\text{PO-LC}}$ represents the AUC for CRA13 (i.e. parent drug excluding metabolites) in plasma following per-oral administration to lymph-cannulated greyhound dogs. For the purpose of this estimation, the approximation has been made that the clearance (mL min^{-1} kg^{-1}) and volume of distribution (L/kg) of CRA13 were the same in greyhounds as in beagles. The absolute bioavailability of CRA13 following oral administration of CRA13 to fed greyhound dogs (F_{total}) was calculated from the sum of F_{lymph} and $F_{\text{systemic blood}}$

Statistical Analysis. In beagle and greyhound studies, statistically significant differences were determined by ANOVA followed by Tukey's test for multiple comparisons at a significance level of $\alpha=0.05$. In the human studies, statistically significant differences at the 5% level were determined by calculating two sided 90% confidence intervals for the ratio of log transformed $\text{AUC}_{0-\text{tz}}$ and $C_{\max}$ data in fasted and fed human subjects. Values outside of the [0.8, 1.25] interval were considered statistically different. Statistical analysis was performed using SPSS for Windows version 15.0 (SPSS Inc, Chicago, IL).

RESULTS

The Effect of Food on the Oral Bioavailability of CRA13 in Healthy Volunteers

The geometric mean plasma-concentration time profiles following oral administration of CRA13 (15 mg) to fasted and fed human volunteers are given in Fig. 2. The summary pharmacokinetic parameters are shown in detail in Table II. Food intake significantly ($p<0.05$) increased the average $C_{\max}$ and $\text{AUC}_{0-\text{tz}}$ of CRA13 (2.8 and threefold, respectively). For $\text{AUC}_{0-\infty}$ data pairs were available for two subjects only, however, $\text{AUC}_{0-\infty}$ increased 4.3 and 4.9-fold in these subjects.

Table II. Single Dose Pharmacokinetics in Healthy Human Volunteers After Oral Administration of 15 mg CRA13 to Fasted and Fed (High-Fat Content) Subjects as a Solidified Micellar Solution in a Hard Gelatin Capsule ($n=12$ for fasted and $n=6$ for fed)

PK parameter	Fasted	Fed
$\text{AUC}_{0-\text{tz}}$ (ng h^{-1} mL^{-1})	525±183	1555±367[c]
$\text{AUC}_{0-\infty}$(ng h^{-1} mL^{-1})	232, 432[b]	1582±413[d]
$C_{\max}$ (ng/mL)	84.4±26.1	246.8±56.0[c]
$T_{\max}$ (h)[a]	2.00 (1.02–3.00)	3.50 (2.00–6.00)
Apparent $t_{1/2}$ (h)	5.00±1.87	13.3±2.7[c]

Data are mean±SD (median for $T_{\max}$)
[a] Data are median values with ranges given in brackets
[b] Individual data for $n=2$ subjects (not reliably characterized in other volunteers due to limited concentration data above the LOQ during the terminal elimination phase)
[c] Values significantly greater when administered in the fed rather than fasted state, $p<0.05$.
[d] $n=5$, not reliably characterised in other volunteer

Determination of the Extent of Absorption and First Pass Metabolism of CRA13 in Beagle Dogs

Fig. 3 shows the systemic plasma concentration-time profiles following IV and oral administration of 1 mg/kg CRA13 to fasted male beagle dogs. Table III gives the corresponding pharmacokinetic parameters. The plasma clearance (Cl) and volume of distribution (V_{D}) of CRA13 in beagles were 402 ml h^{-1} kg^{-1} and 1.0 L/kg respectively. Table IV shows the estimated extent of absorption of CRA13 and its metabolites (calculated according to Eq. 3 using total ^{3}H), the

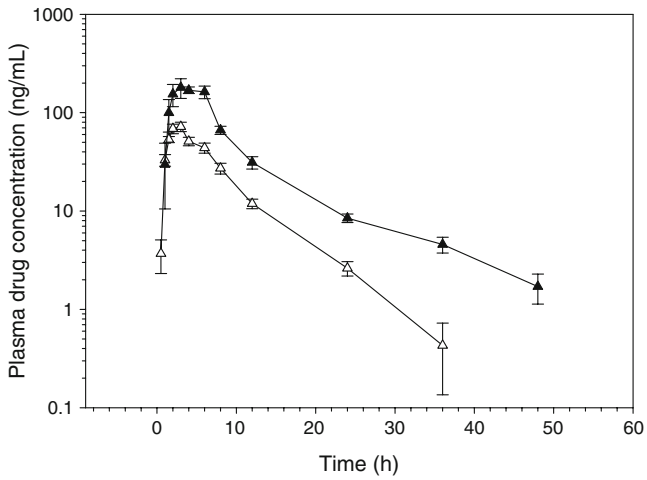

Fig. 2. Systemic plasma concentration-time profiles of CRA13 after oral administration to fed (*filled triangle*) and fasted (*empty triangle*) human volunteers. Data presented as mean±SEM ($n=12$ for fasted and $n=6$ for fed).

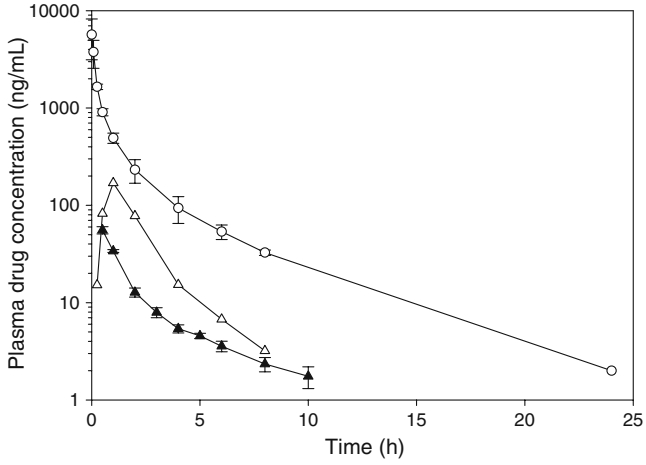

Fig. 3. Systemic plasma concentration-time profiles of CRA13 after IV administration to overnight-fasted male beagle dogs (*circle*, mean± SEM, $n=3$) and after oral administration to overnight-fasted male beagle dogs (*empty triangle*, mean, $n=2$) or post-prandial thoracic lymph duct-cannulated greyhounds (*filled triangle*, mean±SEM, $n=3$). All dogs were dosed at ~1 mg/kg.

Table III. Single Dose Pharmacokinetics of CRA13 After IV and Oral Administration (1 mg/kg) to Fasted Beagles and After Oral Administration (1 mg/kg) to Fed Thoracic Lymph-Duct Cannulated Greyhound Dogs

PK parameter	Beagles		Lymph-cannulated greyhounds
	IV administration (n=3)	Oral administration (n=2)	Oral administration (n=3)
$AUC_{0-\infty}$ (ng h^{-1} mL^{-1})	2,725±358	231.4/438.0	105.0±10.5
C_{max} (ng/mL)	–	87.5/252.7	55.7±4.8
T_{max} (h)	–	1.0/1.0	0.5±0.0
Apparent $t_{1/2}$ (h)	4.0±0.1[a]	1.7/1.8	3.6±0.5
CL (mL h^{-1} kg^{-1})	402±64	–	–
V_D (L/kg)	1.0±0.3	–	–

Data are presented as mean±SD

[a] Data represents n=2 dogs. The apparent terminal elimination half- lives for beagles 1 and 2 were 4.0 and 4.1 hr whereas that of beagle 3 was 24 h. In beagle 3, the plasma concentrations of CRA13 remained above the LOQ for 48 h post-dose whereas for beagle 1 and 2, the plasma concentrations of CRA13 were below the LOQ after the 24 h blood sample. Plasma concentrations of CRA13 appeared to decline in a multiexponential manner in all dogs. The longer terminal elimination half-life of dog 3 most probably reflects the true elimination half-life for CRA13, however, the contribution of this phase of elimination to the total systemic elimination of CRA13 is minor since the fraction of AUC associated with the terminal phase amounts to less than 5% of the total AUC

absolute bioavailability of CRA13 (calculated according to Eq. 4 using parent CRA13 determined by LC-MS/MS) and the estimated extent of first-pass metabolism of CRA13 (based on the difference between the apparent extent of absorption of ^{3}H CRA13 and its metabolites and the absolute bioavailability of CRA13) following oral administration of CRA13 to fasted beagle dogs.

The estimated extent of absorption of ^{3}H-CRA13 and ^{3}H-metabolites from the intestine into the systemic circulation was relatively high in both dogs even after fasted administration (72–75% of the administered dose). By comparison, the absolute bioavailability of CRA13 was low (8–20% of the administered dose). The data therefore suggest that the oral bioavailability of CRA13 in fasted beagle dogs is limited in large part by extensive first pass metabolism (72–89% of the total amount absorbed), rather than poor absorption.

Assessment of CRA13 Lymphatic Transport in Greyhound Dogs

Fig. 4 shows the rate and cumulative extent of transport of triglyceride and CRA13 into thoracic lymph after oral administration of two capsules, each containing 15 mg CRA13 (approximately 1 mg/kg), to fed thoracic lymph duct-cannulated greyhound dogs. A substantial proportion of the administered dose of CRA13 was recovered in the thoracic lymph (panel d—43.7% of the dose) over the 10 h post dose period and lymphatic drug transport was extremely rapid, with the majority of the absorption occurring in the first 2 h following administration. The maximal transport rate of drug and triglyceride largely coincided, however, high transport rates of triglyceride were maintained for longer periods (approximately 2–4 h).

The plasma concentration-time profile following oral administration of 1 mg/kg CRA13 to fed thoracic lymph duct-cannulated greyhounds is shown in Fig. 3 together with the profiles obtained after IV and oral administration of 1 mg/kg CRA13 to fasted beagles. Pharmacokinetic parameters based on these profiles are presented in Table III, and the relative contributions of lymphatic and portal absorption to the total oral bioavailability of CRA13 in fed greyhounds and fasted beagles are presented in Table V. Following oral administration to lymph-cannulated fed greyhound dogs, 43.7% of the 1 mg/kg dose of CRA13 was recovered intact, i.e. not metabolised, in the thoracic lymph (F_{lymph}). In the same animals, the proportion of the dose that was absorbed via the portal blood ($F_{systemic\ blood}$) was estimated to be 3.9%. Whilst there are limitations with the cross study (and breed) comparisons utilised here to estimate absorption into the blood in the lymph cannulated greyhounds, it is apparent that the time to maximum plasma concentration (T_{max}) was similar following oral administration of CRA13 to the beagle and greyhound dogs, suggesting that the rate of absorption was similar across species. Furthermore, the $T_{1/2}$ in lymph-cannulated greyhounds (3.6±0.5 h) was highly comparable with that in beagles that received CRA13 via the IV route (4.0±0.1 h). The overall extent of absorption into the blood was also low. As such, it seems likely that any errors associated with the cross study estimates are unlikely to lead to significant differences in the conclusions drawn—i.e. that absorption directly into the blood was low in fed animals. Following oral administration to fed greyhound dogs, the estimated total oral bioavailability (F_{total}) of CRA13 was therefore 47.5% [i.e. the sum of 3.9% (blood) and 43.7% (lymph)] and transport via the intestinal lymphatic system

Table IV. The Extent of Absorption of CRA13, the Absolute Systemic Bioavailability of CRA13 (F_{total}) and the Estimated Extent of First-Pass Metabolism of CRA13, All Expressed as the Percentage of a 1 mg/kg dose of CRA13 Administered Orally to Two Fasted Beagle Dogs

	Fasted beagle 1	Fasted beagle 2
Extent of absorption (% dose)	75	72
F_{total} (% dose)	8	20
Estimated extent of first-pass metabolism (%)	89	72

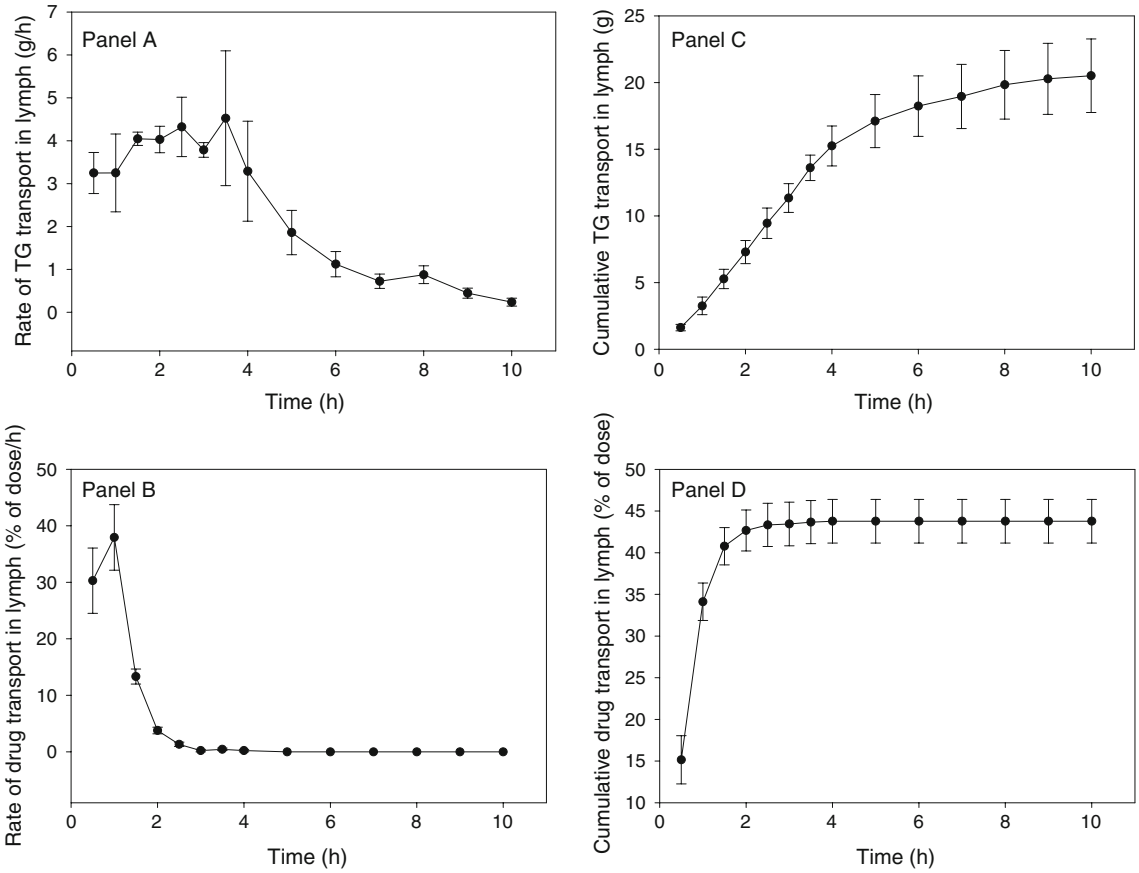

Fig. 4. Rate (g/h or % of dose/h) and cumulative (g or % of dose) thoracic lymphatic transport of TG (**A**, **C**) and CRA13 (**B**, **D**) respectively, following oral administration of ~1 mg/kg CRA13 to post-prandial lymph duct cannulated dogs. Data presented as mean±SEM (n=3).

was responsible for 91.8±0.2% of the oral bioavailability of CRA13.

DISCUSSION

CRA13 is a novel cannabinoid receptor agonist with a physicochemical profile (i.e. high lipophilicity) that is consistent with the involvement of lymphatic transport in the post-prandial absorption profile. The aim of the current study was to first establish whether food had a positive effect on the absorption of CRA13 following oral administration to human volunteers and subsequently to determine the importance of lymphatic transport in any post-prandial increase in bioavailability. Since it is not possible to examine lymphatic transport in humans, the likely extent of lymphatic transport in humans was estimated by examining the extent of intestinal lymphatic transport of CRA13 in greyhound dogs (23). The same formulation was administered in the human studies and lymphatic transport studies in greyhound dogs, allowing direct comparison of the lymphatic transport data in dogs with the effect of food on oral bioavailability in humans. A further study was also performed using radiolabelled drug to facilitate determination of the extent of absorption (in addition to bioavailability) and first pass metabolism of CRA13. The latter study allowed an estimation of the mechanism of post-prandial bioavailability enhancement for CRA13 and in particular the potential for increases in lymphatic transport to lead to a reduction in first pass metabolism.

In human volunteers, overall systemic exposure increased substantially (approximately threefold) when

Table V. The Systemic Availability of a 1 mg/kg Oral Dose Of CRA13 Expressed as a Percentage of the Administered Dose Arising from Lymphatic Transport (F_{lymph}) and Portal Blood Transport ($F_{systemic\ blood}$) in Thoracic Lymph-Duct Cannulated Greyhound Dogs, and as the Total Transport (F_{total}) into the Systemic Circulation in Both Thoracic Lymph-Duct Cannulated Greyhound Dogs and Beagles

Availability of CRA13	Fed lymph-cannulated greyhounds (mean±SD, n=3)	Fasted beagles (individual values, n=2)
F_{lymph} (% dose)	43.7±4.2	n.d.
$F_{systemic\ blood}$ (% dose)	3.9±0.2	n.d.
F_{total} (% dose)	47.5±4.2	8/20
Extent of absorption (% dose)	63.2[a]	75/72

Greyhounds were fed prior to dosing whereas beagles were fasted overnight

[a] Calculated from the sum of the extent of lymphatic transport and absorption via the blood and assuming 80% first pass drug metabolism for the drug recovered in the systemic blood

CRA13 was administered with food rather than in the fasted state. Interestingly, the fasted oral bioavailability of CRA13 was also essentially the same regardless of administration of the solidified micellar formulation described here or as a microemulsion comprising 15 mg of CRA13, 94.5 mg of Cremophor RH40, 13.5 mg propylene glycol and 27 mg Labrafil M 2125 CS diluted with 150 ml of deionized water before dosing (data not shown). Consistent with the lack of formulation effect, subsequent studies using radiolabelled CRA13 suggested that the absorption of CRA13 in beagle dogs, was relatively high (72–75%) even in the fasted state, and that the low bioavailability (8–20%) likely reflected extensive first pass metabolism. Comparison of the extent of absorption and bioavailability of CRA13 suggest that approximately 80% of the absorbed dose was eliminated via first pass metabolism. The complexity of the metabolic pathways of CRA13 precludes detailed discussion here, however in summary, CRA13 appears to be exclusively and extensively eliminated in dogs by oxidative metabolism of the pentyl side chain (by hydroxylation and depentylation) with several oxidized metabolites and at least one *O*-sulfated metabolite being observed in plasma. CRA13 is predominantly excreted in bile with only 1–3% of the dose being recovered in urine after IV and per-oral administration (Novartis data on file).

Blood flowing from the intestine via the portal vein is transported to the liver before entering the systemic blood circulation. In contrast, drugs entering the mesenteric lymph are directly transported to the systemic circulation without first passing through the liver. As such, augmentation of drug uptake into the lymph reduces the opportunity for first pass metabolism to limit oral bioavailability. Indeed, stimulation of lymphatic transport has been shown previously to enhance the bioavailability of drugs (e.g. testosterone) (9) where significant hepatic first pass metabolism is a limitation to (or in some cases precludes) oral bioavailability (10). A recent study has also suggested that stimulating lymphatic transport by administration of lipid may protect drugs from enterocyte-based metabolism by altering the manner in which lipophilic drugs partition within the enterocytes thereby reducing drug access to metabolic enzymes (8).

In fed greyhound dogs, 43.7% of the administered dose of CRA13 was transported into the intestinal lymph (Table V). This represents a significant proportion of the absorbed dose irrespective of that absorbed via the portal vein. However, a broad estimate of the extent of drug transport to the systemic circulation via the blood can be made by comparison of the plasma-concentration time profile obtained after oral administration to the lymph-cannulated greyhounds with the IV data obtained in beagles. This is an imperfect comparison and provides only an indication of the likely extent of systemic exposure via portal absorption, however, the similarities in terminal half life between the two groups provide some degree of confidence that clearance and volume of distribution were not markedly different across groups (i.e. between breeds). Acknowledging this caveat, comparison of these data sets suggests that absorption via the portal blood resulted in only 3.9% systemic bioavailability. Assuming approximately 80% first pass metabolism, this suggests that approximately 20% of the dose of CRA13 was absorbed into the portal blood (to provide 3.9% systemic availability post first pass) and that the total percent absorbed (i.e. via the portal blood and intestinal lymph) was of the order of 63% (Table V). This is reasonably consistent with the extent of absorption as measured using total radioactivity in fasted beagle dogs (70–75%). Post-prandial administration therefore does not appear to increase the extent of absorption, but rather redirects a significant proportion of the absorbed dose to the intestinal lymph which in turn results in a reduction in first-pass metabolism and an increase in bioavailability. It seems likely, therefore, that the pronounced food effect on oral bioavailability in humans is also due to enhanced drug transport via the intestinal lymph and avoidance of first-pass metabolism following post-prandial administration rather than an increase in drug absorption *per se*.

CONCLUSION

CRA13 is a novel, lipophilic cannabinoid agonist. In the current studies, CRA13 was substantially transported (47.5% of the dose) to the systemic circulation via the intestinal lymphatic system when administered orally to post-prandial greyhound dogs. The absolute bioavailability of CRA13 was approximately 50% in the same animals suggesting that greater than 90% of the systemic exposure resulted from lymphatic transport. In contrast, when administered to fasted dogs, the absolute oral bioavailability of CRA13 was relatively low with only 8–20% of the parent drug appearing in the systemic circulation. The poor bioavailability of CRA13 in fasted dogs does not appear to reflect poor absorption, however, but rather extensive first-pass metabolism. Administration of CRA13 with food therefore enhances oral bioavailability by stimulating lymphatic drug transport which in turn provides a transport route to the systemic circulation that avoids first-pass metabolism. The substantial effect of food on the oral bioavailability of CRA13 in humans is, therefore likely to reflect enhanced lymphatic transport and avoidance of first-pass metabolism in the fed state.

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REFERENCES

1. C. J. H. Porter, N. L. Trevaskis, and W. N. Charman. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat. Rev. Drug. Discov.* **6**:231–248 (2007). doi:10.1038/nrd2197.
2. C. M. O'Driscoll. Lipid-based formulations for intestinal lymphatic delivery. *Eur. J. Pharm. Sci.* **15**:405–415 (2002). doi:10.1016/S0928-0987(02)00051-9.
3. D. M. Karpf, R. Holm, H. G. Kristensen, and A. Mullertz. Influence of the type of surfactant and the degree of dispersion on the lymphatic transport of halofantrine in conscious rats. *Pharm. Res.* **21**:1413–1418 (2004). doi:10.1023/B:PHAM.0000036915.03725.19.
4. A. Dahan, R. Duvdevani, I. Shapiro, A. Elmann, E. Finkelstein, and A. Hoffman. The oral absorption of phospholipid prodrugs: *in vivo* and *in vitro* mechanistic investigation of trafficking of a lecithin-valproic acid conjugate following oral administration. *J.*

Control Release. **126**:1–9 (2008). doi:10.1016/j.jconrel.2007.10.025.

5. A. Dahan, A. Mendelman, S. Amsili, N. Ezov, and A. Hoffman. The effect of general anesthesia on the intestinal lymphatic transport of lipophilic drugs: comparison between anesthetized and freely moving conscious rat models. *Eur. J. Pharm. Sci.* **32**:367–374 (2007).
6. P. Gershkovich, and A. Hoffman. Effect of a high-fat meal on absorption and disposition of lipophilic compounds: the importance of degree of association with triglyceride-rich lipoproteins. *Eur. J. Pharm. Sci.* **32**:24–32 (2007). doi:10.1016/j.ejps.2007.05.109.
7. D. J. Hauss, S. C. Mehta, and G. W. Radebauch. Targeted lymphatic transport and modified systemic distribution of CI-976, a lipophilic lipid-regulator drug, via a formulation approach. *Int. J. Pharm.* **108**:85–93 (1994). doi:10.1016/0378-5173(94)90318-2.
8. N. L. Trevaskis, C. J. H. Porter, and W. N. Charman. An examination of the interplay between enterocyte-based metabolism and lymphatic drug transport in the rat. *Drug Metab. Dispos.* **34**:729–733 (2006). doi:10.1124/dmd.105.008102.
9. D. M. Shackleford, W. A. Faassen, N. Houwing, H. Lass, G. A. Edwards, C. J. H. Porter, and W. N. Charman. Contribution of lymphatically transported testosterone undecanoate to the systemic exposure of testosterone after oral administration of two andriol formulations in conscious lymph duct-cannulated dogs. *J. Pharmacol. Exp. Ther.* **306**:925–933 (2003). doi:10.1124/jpet.103.052522.
10. S. M. Khoo, G. A. Edwards, C. J. H. Porter, and W. N. Charman. A conscious dog model for assessing the absorption, enterocyte-based metabolism, and intestinal lymphatic transport of halofantrine. *J. Pharm. Sci.* **90**:1599–1607 (2001). doi:10.1002/jps.1110.
11. S. M. Sieber, V. H. Cohn, and W. T. Wynn. The entry of foreign compounds into the thoracic duct lymph of the rat. *Xenobiotica.* **4**:265–284 (1974).
12. A. Vost, and N. Maclean. Hydrocarbon transport in chylomicrons and high-density lipoproteins in rat. *Lipids.* **19**:423–435 (1984). doi:10.1007/BF02537404.
13. C. T. Phan, and P. Tso. Intestinal lipid absorption and transport. *Front Biosci.* **6**:D299–319 (2001). doi:10.2741/Phan.
14. B. K. Nordskog, C. T. Phan, D. F. Nutting, and P. Tso. An examination of the factors affecting intestinal lymphatic transport of dietary lipids. *Adv. Drug. Deliv. Rev.* **50**:21–44 (2001). doi:10.1016/S0169-409X(01)00147-8.
15. C. J. H. Porter, and W. N. Charman. Intestinal lymphatic drug transport: an update. *Adv. Drug Deliv. Rev.* **50**:61–80 (2001). doi:10.1016/S0169-409X(01)00151-X.
16. W. N. Charman, and V. J. Stella. Estimating the maximum potential for intestinal lymphatic transport of lipophilic drug molecules. *Int. J. Pharm.* **34**:175–178 (1986). doi:10.1016/0378-5173(86)90027-X.
17. C. A. Lipinski. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods.* **44**:235–249 (2000). doi:10.1016/S1056-8719(00)00107-6.
18. S. M. Khoo, D. M. Shackleford, C. J. H. Porter, G. A. Edwards, and W. N. Charman. Intestinal lymphatic transport of halofantrine occurs after oral administration of a unit-dose lipid-based formulation to fasted dogs. *Pharm. Res.* **20**:1460–1465 (2003). doi:10.1023/A:1025718513246.
19. E. K. Dziadulewicz, S. J. Bevan, C. T. Brain, P. R. Coote, A. J. Culshaw, A. J. Davis, L. J. Edwards, A. J. Fisher, A. J. Fox, C. Gentry, A. Groarke, T. W. Hart, W. Huber, I. F. James, A. Kesingland, L. La Vecchia, Y. Loong, I. Lyothier, K. McNair, C. O'Farrell, M. Peacock, R. Portmann, U. Schopfer, M. Yaqoob, and J. Zadrobilek. Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl) methanone: a potent, orally bioavailable human CB1/CB2 dual agonist with antihyperalgesic properties and restricted central nervous system penetration. *J. Med. Chem.* **50**:3851–3856 (2007). doi:10.1021/jm070317a.
20. C. C. Felder, A. K. Dickason-Chesterfield, and S. A. Moore. Cannabinoids biology: the search for new therapeutic targets. *Mol. Interv.* **6**:149–161 (2006). doi:10.1124/mi.6.3.6.
21. G. T. Whiteside, G. P. Lee, and K. J. Valenzano. The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of small molecule CB2 receptor agonists. *Curr. Med. Chem.* **14**:917–936 (2007). doi:10.2174/092986707780363023.
22. A. Lespine, G. Chanoit, A. Bousquet-Melou, E. Lallemand, F. M. Bassissi, M. Alvinerie, and P. L. Toutain. Contribution of lymphatic transport to the systemic exposure of orally administered moxidectin in conscious lymph duct-cannulated dogs. *Eur. J. Pharm. Sci.* **27**:37–43 (2006). doi:10.1016/j.ejps.2005.08.003.
23. G. A. Edwards, C. J. H. Porter, S. M. Caliph, S. M. Khoo, and W. N. Charman. Animal models for the study of intestinal lymphatic drug transport. *Adv. Drug Deliv. Rev.* **50**:45–60 (2001). doi:10.1016/S0169-409X(01)00148-X.
24. T. T. Kararli. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos.* **16**:351–380 (1995). doi:10.1002/bdd.2510160502.
25. N. L. Trevaskis, C. J. H Porter, and W. N. Charman. Bile increases intestinal lymphatic drug transport in the fasted rat. *Pharm. Res.* **22**:1863–1870 (2005). doi:10.1007/s11095-005-6808-9.
26. FDA, Center for Drug Evaluation and Research (CDER). Guidance for industry: food-effect bioavailability and fed bioequivalence studies. US, 2002.