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Research Paper

Bile Increases Intestinal Lymphatic Drug Transport in the Fasted Rat

Natalie L. Trevaskis,[1] Christopher J. H. Porter,[1,2] and William N. Charman[1]

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Purpose. This study was conducted to determine the influence of (1) the source of recruited endogenous fatty acid (FA), and (2) bile on intestinal lymphatic transport of halofantrine (Hf) in fasted rats.
Methods. Endogenous FA output in bile, and exogenous (^{14}C radiolabeled) FA, endogenous FA, and Hf transport in mesenteric lymph were determined following administration of low dose lipid formulations containing either 4 or 40 mg of exogenous FA [oleic acid (OA)] and different amounts of bile salt (BS) and lysophosphatidylcholine (LPC) to fasted rats.
Results. Administration of 40 mg of OA recruited endogenous FA and Hf transport into intestinal lymph, whereas 4 mg OA did not. However, addition of BS to the 4-mg OA dose led to stimulation of endogenous FA recruitment into lymph and an increase in lymphatic transport of Hf and endogenous FA output in bile. Addition of LPC to the 4-mg OA dose (dispersed in BS) caused a substantial increase in endogenous FA transport in lymph; however, no coincident increase in either lymphatic transport of Hf or endogenous FA output in bile was observed.
Conclusion. Biliary-derived endogenous FA has a higher propensity to support lymphatic transport of Hf compared to other sources of endogenous FA. The results suggest that this is related to the disparate trafficking of these alternate sources of endogenous FA within the enterocyte.

KEY WORDS: bile; drug absorption; lipid-based formulations; lymphatic transport; poorly water-soluble drugs.

INTRODUCTION

The products of dietary lipid digestion are absorbed from the intestinal lumen into the enterocyte leading to either absorption via the portal vein *per se*, or cytoplasmic transport to the smooth endoplasmic reticulum (SER) (1), where they are reesterified prior to incorporation into lipid-rich lipoproteins (LP). The lipid-rich LP are then exocytosed across the basolateral membrane of the enterocyte into the lamina propria and transported via the intestinal lymphatic system into the systemic circulation. Although LP formation and intestinal lymph lipid transport are significantly increased in the fed state, substantial endogenous lipid (in the form of FA attached to triglyceride and phospholipid) flux into intestinal lymph is maintained under fasting conditions (2–6).

Endogenous FA enter the enterocyte from either the luminal (apical) or basolateral side of the cell (2,7,8). Apically sourced endogenous FA includes the FA output in bile (associated with phospholipid transport) and those arising from desquamated enterocytes, whereas basolaterally sourced endogenous FA comes from the uptake of FA (9) and chylomicron (CM) remnants from the intestinal blood supply (10). A further potential source of endogenous FA is *de novo* synthesis within the enterocyte (2,7,8).

The primary source (~50%) of endogenous FA in fasted intestinal lymph is bile, with biliary diversion leading to a substantial reduction in endogenous FA transport in lymph (11–13). Cell desquamation has been estimated to only contribute up to 1 mg/h of endogenous lipid (2), and there is little *de novo* FA synthesis occurring within the enterocyte (2,7,8). Although a proportion of the basolaterally sourced endogenous FA entering the enterocyte may be lymphatically transported, the majority of these enter a cytosolic lipid pool leading to transport via the portal vein (9).

Biliary components also facilitate the absorption of dietary lipid digestion products by enhancing their solubilization in the intestinal milieu in the form of bile salt (BS)–phospholipid (PL) mixed micelles and vesicular colloids (1,14). Biliary-derived PL is also thought to be necessary to support postprandial formation of lipid-rich LP (such as CM) (11,15,16). In support of this hypothesis, the addition of PL to intraduodenal lipid infusions of triolein and BS has been shown to substantially enhance lymph lipid transport (11–13,17–19).

Similar to lipids, drugs may be absorbed into either the portal vein and/or intestinal lymphatics after oral administration. However, only highly lipophilic drugs (usually log $p > 5$; triglyceride solubility > 50 mg/g) are substantially ab-

[1] Department of Pharmaceutics, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia.
[2] To whom correspondence should be addressed. (e-mail: chris.porter@vcp.monash.edu.au)

ABBREVIATIONS: BS, bile salt; CM, chylomicron; FA, fatty acid; Hf, halofantrine; LP, lipoprotein; LPC, lysophosphatidylcholine; OA, oleic acid; PL, phospholipids; SER, smooth endoplasmic reticulum; TG, triglyceride.

sorbed via the intestinal lymphatic system, with transport typically being a consequence of their association with lipid-rich LP (20). Because lipid and drug transport in lymph are closely related (20,21), absorption of drugs via the intestinal lymphatic system has generally been assumed to require coadministration with a meal to stimulate LP formation and subsequently lipid and drug transport in lymph (20,21). However, a recent study from our laboratory has demonstrated substantial lymphatic drug transport in the fasted state (22) after administration of a single unit dose lipid-based formulation to fasted dogs. In this previous study, the fasted state lymphatic transport of drug was apparently supported by the recruitment of endogenous FA transport into lymph (22).

Lymph lipid and drug transport are therefore interrelated and endogenous FA, the major source of lymph lipids in the fasted state, might be expected to influence fasted state lymphatic drug transport. In this study, (1) the influence of bile on fasted state lymphatic transport of halofantrine (Hf, a model lipophilic drug) was examined, and more specifically, (2) the importance of the source of recruited endogenous FA (biliary-derived or blood-derived) in stimulating intestinal lymphatic transport of Hf, was assessed. The study involved a comparison of endogenous FA output in bile, lymphatic transport of endogenous FA, and lymphatic transport of Hf following the systematic addition of bile components [BS and lysophosphatidylcholine (LPC)] to an intraduodenal infusion containing a small amount of exogenous FA (4 mg) when administered to fasted rats. The results indicate that bile-derived endogenous FA have a greater propensity to support lymphatic transport of Hf than basolaterally sourced endogenous FA.

MATERIALS AND METHODS

Materials

Halofantrine base (Hf) (GlaxoSmithKline, Mumbai, India), oleic acid [1-^{14}C] (Perkin Elmer Life Sciences, Boston, MA, USA), oleic acid (OA), L-α-lysophosphatidylcholine (LPC) and taurocholate (Sigma, Perth, Australia), Tween 80 (BDH Chemicals, Kilsyth, Victoria, Australia), and normal saline (NS) for injection (Baxter Healthcare, Sydney, Australia) were used as received. Acetonitrile, sodium dodecyl sulfate, and glacial acetic acid were HPLC-grade. Water was obtained from a Milli-Q (Millipore, Milford, MA, USA) purification system. Hypnovel® (Sigma) and Hypnorm® (Vet Drug, York, UK) were used for anesthesia. TG kit®, PL kit®, Control for automated systems®, and Precinorm U® (Roche Diagnostics, Indianapolis, IN, USA) were used for analysis of triglyceride (TG) and phospholipid (PL) levels. Starscint® (Packard Bioscience, Meriden, CT, USA) liquid scintillation cocktail was used for liquid scintillation counting of radioactivity levels. All other chemicals were analytical reagent grade.

Composition of Lipid Formulations

A standard 4-mg dose of exogenous OA (a type of FA) was selected for administration to rats as it approximates, on a mg/kg basis, a 1-g lipid-based formulation typically administered to humans. A higher 40-mg dose of exogenous OA was also selected for administration to rats to determine whether the higher lipid dose would recruit more endogenous FA transport into lymph than the 4-mg dose. A 10.4-mg dose of LPC was chosen because it is similar to the amounts of PC previously reported to enhance lymph lipid transport after intraduodenal administration (12,13,23). The rate of BS administration (14 μmol/h of BS over 2 h) was similar to the bile-derived physiological input rate into the rat intestine (10–20 μmol/h) under fasted conditions (24). Tween 80, a surfactant commonly used in oral formulations, was chosen to emulsify the exogenous lipid formulations where administration in the absence of BS was required.

Preparation of Lipid Formulations

^{14}C OA (5 μCi) and Hf (200 μg) were incorporated in the lipid phase (4 mg OA, 40 mg OA, or 4 mg OA/10.4 mg LPC) of formulations prior to dispersion in different aqueous phases. The two aqueous phases studied were 0.2% (w/v) Tween 80 in normal saline (pH 7.0) or a bile salt solution (BS solution) comprising 5 mM sodium taurocholate in phosphate buffer (pH 6.9). The formulations were emulsified by ultrasonification with a Misonix XL 2020 ultrasonic processor (Misonix, Farmingdale, NY) equipped with a 3.2-mm microprobe tip running at an amplitude of 240 μm and a frequency of 20 kHz for 2 min at room temperature. The Hf concentration was verified using a validated HPLC assay (25) on the day of dosing, and the chemical and physical stability of the formulations were monitored throughout the study. Particle size analysis was performed using a Malvern Zetasizer 3000 equipped with a 5-mW He–Ne laser at 633 nm (Malvern Instruments Ltd., Malvern, UK). The particle size of all formulations was monitored for 48 h to ensure no change occurred prior to dosing.

Experimental Design

In Part 1 of the study, formulations containing the exogenous FA (OA) were infused intraduodenally for 2 h to lymph-cannulated, fasted, anesthetized rats. These formulations consisted of either (1) 4 mg OA dispersed in 0.2% Tween 80, (2) 4 mg OA dispersed in the BS solution, (3) 4 mg OA/10.4 mg LPC dispersed in the BS solution, (4) 40 mg OA dispersed in 0.2% Tween 80, or (5) normal saline (as a control). Lymph was collected for 10 h following commencement of formulation dosing and endogenous and exogenous (^{14}C labeled) FA and Hf transport in lymph was determined. This enabled assessment of the effect of biliary components (BS and LPC) on endogenous FA and Hf transport in intestinal lymph and assessment of the effect of exogenous FA dose (4 or 40 mg OA) on endogenous FA recruitment.

In Part 2 of the study, nonlymph cannulated, fasted, anesthetized rats were similarly infused intraduodenally over 2 h with exogenous FA formulations containing either (1) 4 mg OA dispersed in 0.2% Tween 80, (2) 4 mg OA dispersed in the BS solution, (3) 4 mg OA/10.4 mg LPC dispersed in the BS solution, or (4) normal saline as a control. At 2 h 50 min after commencement of the intraduodenal infusion (i.e., 50 min after completion of administration of the lipid containing infusion), the common bile duct in each rat was

cannulated to enable collection of bile during the subsequent 2-h period (i.e., 3–5 h after initiation of the intraduodenal infusion of formulation). This experimental design ensured that the majority of the formulation had been absorbed prior to collection of bile and enabled determination of the effect of exogenous BS and LPC on endogenous FA output in bile.

Overall, this study design enabled comparison of endogenous FA and exogenous FA and Hf transport in lymph (Part 1) with endogenous FA output in bile (Part 2). This comparison was performed so that the source of the endogenous FA (bile or nonbile) that were recruited to support the formulation-dependent increases in lymph lipid and Hf transport could be determined.

Choice of Animal Model

Recent studies from our laboratory have advocated the utility of canine models for the examination of intestinal lymphatic drug transport (20,22,28,29). Although this remains an ideal model with which to probe the extent of lymphatic transport after the administration of a particular drug product (because it allows administration of full-scale clinical dose forms), there are considerable ethical and logistical limitations associated with the conduct of multiple mechanistic studies in dogs. As a result, the current studies were conducted in the rat, because this allows examination of a considerably larger number of experimental variables. Studies in the rat also allow comparison with a substantial historical database describing the biochemistry of lipid absorption and intracellular processing, a database that is not available in the dog. An anesthetized (rather than conscious) rat model was employed because experiments can be conducted more quickly without the requirement for surgical recovery and because it is easier to maintain the patency of lymph duct cannulas in anesthetized, rather than conscious, animals. Anesthesia, however, may reduce gastric emptying and intestinal lipid processing, and as such the animals were dosed intraduodenally (to circumvent the problem of delayed gastric emptying) with pre–emulsified formulations containing the predigested lipids OA and LPC (to prevent complications associated with reduced intestinal processing and digestion). In support of this approach, studies from our laboratory have previously shown that although the intestinal lymphatic transport of Hf may be reduced following the administration of simple lipid solution formulations to anesthetized (rather than conscious) rats, these differences may be circumvented by the intraduodenal administration of predispersed formulations (26,27).

Surgical and Experimental Procedures

All surgical and experimental procedures were approved by the local institutional Animal Ethics Committee. Male Sprague–Dawley rats (280–320 g) were fasted overnight prior to initiation of anesthesia via a 3 mL/kg intraperitoneal (IP) injection of "FFM" containing 1 part Hypnorm® (Fentanyl 0.2 mg/mL, Fluanisone 10 mg/mL; Janssen Animal Health, Petteridge, UK), 1 part Hypnovel® (Midazolam 5 mg/mL; Roche, Sydney, Australia) and 2 parts Milli Q water. Additional IP doses of 0.1–0.4 mL of 1:1 (v/v) Hypnorm®/water were administered every 20–60 min depending on the depth of anesthesia required, with top-up doses of 0.2–0.4 mL FFM administered every 4 h. The body temperature of the anesthetized rats was maintained by placing them on a heated pad maintained at 37°C (Ratek Instruments, Subiaco, Australia). At the conclusion of the experiment, rats were euthanized via a lethal IP dose of 1 mL sodium pentobarbitone (100 mg/mL).

Part 1: Lymph Cannulated Rats

A tracheal cannulation was performed using a 2-cm piece of polyethylene tubing (2 mm, i.d.) to provide a clear airway. The duodenum was cannulated 1 cm below the pylorus with a J-shaped, heat-molded segment of polyethylene tubing (PE 50, Clay Adams, Parsippany, NJ). The mesenteric lymph duct was cannulated with polyethylene tubing (0.5 mm i.d., 0.8 mm o.d.; Dural Plastics, Huntingdale, Australia) as previously described (21,30). Both the mesenteric lymph duct cannula and duodenal cannula were secured with instant cyanoacrylate adhesive (Selleys Supa Glue®, Padstow, Australia).

The rats were rehydrated at a rate of 2.8 mL/h via the duodenum with normal saline following surgery, a rehydration rate commonly employed in similar studies (3–8) and which provides an appropriate level of hydration during drainage of mesenteric lymph. The animal was allowed to rehydrate for 0.5 h prior to intraduodenal infusion of 5.6 mL (2.8 mL/h over 2 h) of experimental lipid formulation. Following the 2-h dosing period, normal saline was then infused intraduodenally at 2.8 mL/h for the remainder of the experiment. Lymph was continuously collected for 10 h into tared polyethylene tubes containing 100 μL of 1,000 IU/mL heparin. Collection tubes were changed hourly.

Part 2: Bile Duct Cannulated Rats

The duodenum and trachea were cannulated as in Part 1. A continuous intraduodenal infusion of normal saline at 2.8 mL/h was also initiated after completion of surgery, and the animal allowed to rehydrate for 0.5 h prior to intraduodenal infusion of 5.6 mL (2.8 mL/h over 2 h) of experimental lipid formulation. After the 2-h dosing period, normal saline (NS) was infused intraduodenally at 2.8 mL/h for the remainder of the experiment. At 2 h and 50 min after commencement of formulation administration (i.e., 50 min after completion of administration of the lipid containing infusion), the rats were reopened along the abdominal incision and the common bile duct cannulated with polyethylene tubing (0.5 mm i.d., 0.8 mm o.d.; Dural Plastics, Huntingdale, Australia) and secured with surgical suture and a drop of cyanoacrylate adhesive. Collection of bile commenced 3 h after initiation of formulation administration and continued for 2 h. Bile was collected on ice into tared tubes, which were changed hourly.

Analytical Procedures

Halofantrine Transport in Lymph

Lymph concentrations of Hf were determined using a previously validated method for Hf analysis (25). However, the preparation of lymph samples for HPLC analysis was

altered by diluting them either 1:80 (v/v) or 1:40 (v/v) in acetonitrile (depending on assay sensitivity requirements), vortexing for 1 min, centrifuging for 3 min at ~1,500×*g* to remove insoluble components prior to direct injection onto the HPLC. Recovery of Hf spiked into blank lymph (at concentrations of 0.5, 1, and 2 μg/mL) was >95% (n = 5 analyses at each concentration).

Exogenous and Endogenous FA Transport in Lymph and Endogenous FA Output in Bile

Lymph TG, lymph PL, and bile PL concentrations were determined using commercial enzymatic colorimetric methods running on a Cobas Mira lipid analyzer (Roche, Indianapolis, IN), as described previously (28). The TG and PL concentration in lymph were used to calculate total (endogenous plus exogenous) FA transport in lymph (expressed in terms of the total moles of FA, and calculated assuming that each mole of lymph TG and PL comprised 3 and 2 mol of FA, respectively). Similarly, the PL concentration in bile was used to calculate the endogenous FA output in bile by assuming that each mole of PL contained 2 mol of FA.

The quantity of exogenous, radiolabeled FA transported in each hourly lymph sample was measured by scintillation counting following addition of 1 mL Starscint® to a 30-μL lymph sample. The scintillation method was validated by spiking blank lymph samples with known amounts of ^{14}C OA (0.0002–0.05 μCi per 30 μL of lymph) and the measured concentrations were within 5% of the nominal concentration. Endogenous FA transport in intestinal lymph was calculated by subtracting the mass of exogenous FA recovered in lymph each hour from the mass of total FA recovered in lymph each hour.

Statistical Analysis

Results were analyzed using a Dunnett T3 test for multiple comparisons assuming unequal variance (groups did not show equal variance in the Levene homogeneity of variance test) at a significance level of $\alpha = 0.05$. Analyses were performed using SPSS for Windows versions 11.5.0. (SPSS Inc, Chicago, IL, USA).

RESULTS

Formulation Characteristics

The formulations containing 40 mg OA dispersed in 5.6 mL of 0.2% Tween 80 comprised two different-sized populations of lipid droplets with mean diameters of 100–120 and 350–370 nm and which occurred at 66 and 34% frequency by volume, respectively. The formulations containing 4 mg OA dispersed in 5.6 mL of either 5 mM BS solution or 0.2% Tween 80 contained lipid droplet populations with similar size distributions. The droplets had mean diameters of 100–110 and 290–300 nm, occurring at 70–80 and 20–30% frequency by volume, respectively. The formulation containing 4 mg OA/10.4 mg LPC dispersed in 5 mM BS solution contained lipid droplets with mean diameter 25–30 nM. The particle size of all formulations did not change significantly over a period of at least 48 h postpreparation.

Endogenous FA Transport in Lymph

The appearance of endogenous FA associated with TG in intestinal lymph of fasted rats that had received a control intraduodenal infusion of normal saline was constant over the 10-h collection period (10.3 ± 0.6 μmol/h) and similar to results previously reported for similar studies with fasted, anesthetized rats (9.1 ± 1.2 μmol/h) (26). After infusion of 4 mg OA dispersed in 0.2% Tween 80, the endogenous FA transport in lymph (comprising TG FA and PL FA) over the 10-h postdosing period was not significantly different from that observed in the control normal saline group (Table I). These data indicate that an exogenous FA dose of 4 mg was unable to recruit endogenous FA transport into lymph under these conditions. In contrast, the presence of BS resulted in a significant increase (and apparent stimulation) in endogenous FA transport in lymph as either infusion of BS solution alone (instead of normal saline) or dispersion of the 4-mg OA formulation in BS solution significantly enhanced transport over the 10-h postdosing period (Table I and Fig. 1).

The addition of LPC to the 4-mg OA formulation dispersed in BS solution led to a further and significant increase in endogenous FA transport in lymph when compared with either the Tween 80 or BS formulations (Table I and Fig. 1). In all groups infused with 4 mg of OA (equivalent to 14.1 μmol of FA), endogenous FA were the primary form of FA in lymph and contributed >85 μmol FA over the 10-h period, whereas only approximately 50% of the administered exogenous 14.1 μmol (4 mg) OA lipid dose was lymphatically transported (data not shown).

After infusion of 40 mg OA dispersed in 0.2% Tween 80, there was a substantial increase in endogenous FA transport in lymph compared to administration of 4 mg OA emulsified in Tween 80 (Table I). This indicates that the higher 40-mg

Table I. Cumulative Transport of Endogenous Fatty Acid (FA) (μmol) (Comprising Triglyceride and Phospholipid Derived FA) in Mesenteric Lymph Duct Cannulated, Anesthetized Rats (Mean ± SEM, n = 4) Following Intraduodenal Infusion of Different Formulations During the 0–2 h Period

Formulation	Cumulative transport of endogenous FA (μmol) in lymph over 10 h
Normal saline	95.6 ± 4.6
5 mM bile salt solution	167.4 ± 4.1[a]
4 mg oleic acid (OA) dispersed in 0.2% Tween 80 in normal saline	85.2 ± 4.2
4 mg OA dispersed in 5 mM bile salt solution	154.9 ± 2.8[a]
4 mg OA/10.4 mg lysophosphatidylcholine dispersed in 5 mM bile salt solution	231.2 ± 9.7[a,b]
40 mg OA dispersed in 0.2% Tween 80	187.6 ± 7.6[a]

All oleic acid formulations contained 200 μg halofantrine.
[a] Statistically different to both normal saline and 4 mg OA dispersed in 0.2% Tween groups ($p < 0.05$).
[b] Statistically different to 4 mg OA dispersed in 5 mM bile salt solution ($p < 0.05$).

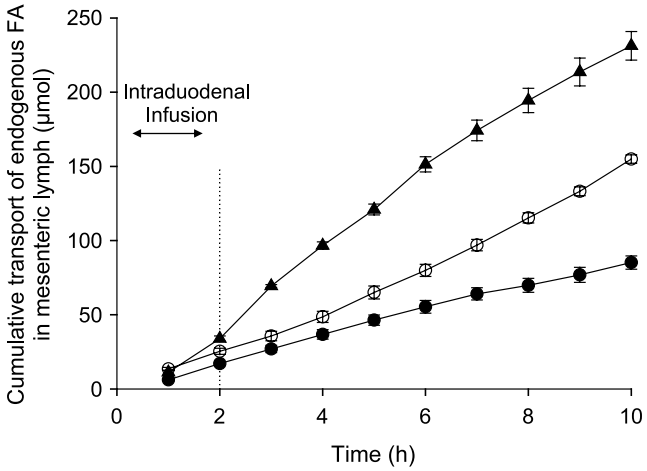

Fig. 1. Cumulative transport of endogenous fatty acid (FA) (comprising triglyceride and phospholipid derived FA) in lymph (μmol) in mesenteric lymph duct cannulated, anesthetized rats (mean ± SEM, $n = 4$) following intraduodenal infusion of formulations from 0 to 2 h. Formulations contained 200 μg halofantrine (Hf) dispersed in either; 4 mg oleic acid (OA) emulsified in 0.2% Tween 80 in normal saline (●); 4 mg OA emulsified in 5 mM bile salt solution (○); or 4 mg OA/10.4 mg lysophosphatidylcholine emulsified in 5 mM bile salt solution (▲).

dose of OA was able to recruit endogenous FA transport into lymph, whereas the 4-mg OA dose was not (Table I).

Endogenous FA Output in Bile

The appearance of endogenous FA in the bile of fasted rats that received a control intraduodenal infusion of normal saline (7.2 ± 0.5 μmol/h) was similar to results reported previously (5.7–8.8 μmol/h) (2,24). For the experimental formulations, infusion of 4 mg OA dispersed in 0.2% Tween 80 did not increase endogenous FA output in bile over the 3- to 5-h period following commencement of formulation infusion; this was consistent with a corresponding lack of change in the endogenous FA transport into lymph over the same 3- to 5-h period (Table II). In contrast, the presence of BS in the 4-mg OA lipid formulation significantly increased endogenous FA output in bile over 3–5 h (3.3-fold) and endogenous FA transport in lymph over 3–5 h (1.5-fold) (Table II). The further addition of LPC to the formulation containing 4 mg OA dispersed in BS solution did not increase endogenous FA output in bile compared to infusion of 4 mg OA dispersed in BS solution (Table II). However, this was in contrast to the endogenous FA transport in lymph over the 3- to 5-h period, which was significantly increased (1.8-fold) following addition of LPC to the formulation containing 4 mg OA dispersed in BS solution (Table II).

Lymphatic Drug Transport

The cumulative transport of Hf in the intestinal lymph over 10 h following intraduodenal infusion of the formulation containing 4 mg OA dispersed in Tween 80 was 7.02 ± 0.5% (Fig. 2). Addition of BS to the 4-mg OA infusion significantly increased the cumulative lymphatic transport of Hf over 10 h by 1.9-fold (to 13.2 ± 0.1%) (Fig. 2), which was consistent with the magnitude of the increased transport of endogenous FA in lymph over 10 h (1.8-fold) (Table I) and an increase in endogenous FA output in bile (Table II). Addition of LPC to the formulation containing 4 mg OA dispersed in BS solution had no significant effect on the cumulative transport of Hf in lymph over 10 h (Fig. 2), reflecting the lack of change in endogenous FA output in bile (Table II) but in contrast to the endogenous FA transport in lymph, which was substantially increased (Fig. 1 and Table I).

DISCUSSION

Lymphatic lipid and drug absorption are closely related because lipophilic drugs are absorbed via the intestinal lymphatic system in association with lipid-rich LP. Lymphatic drug transport has therefore typically been assumed to be negligible except after postprandial administration, with the lipid content of the meal providing the lipid digestion products necessary for synthesis and formation of lipid-rich LPs (20,28). However, substantial lymphatic drug transport in the fasted state was recently demonstrated (22) after administration of a single, unit dose, lipid-based formulation. This substantial fasted state lymphatic drug transport seemed to be supported by recruitment of endogenous FA flux into the lymph. Bile is the major source of endogenous FA in lymph and has previously been shown to facilitate intestinal lymphatic lipid absorption via several mechanisms. As such, the current study aimed to determine whether (1) specific sources of recruited endogenous FA in lymph and (2) bile enhance fasted state lymphatic transport of Hf. Specifically, the study involved comparison of endogenous FA output in bile and endogenous FA and Hf transport in lymph following systematic addition of bile components (BS and LPC) to a

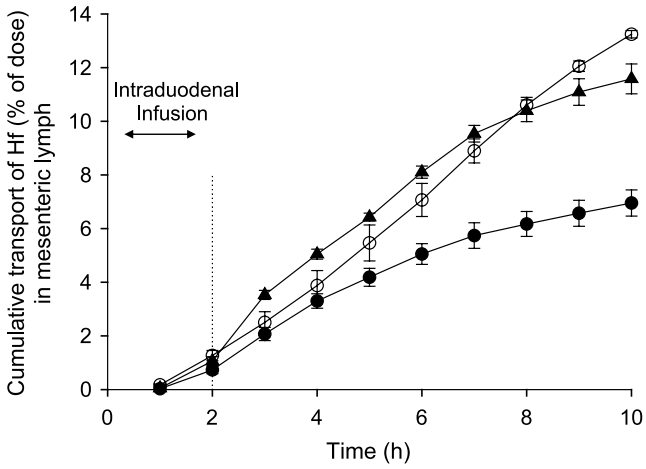

Fig. 2. Cumulative transport of halofantrine (% dose Hf) in lymph in mesenteric lymph duct cannulated, anesthetized rats (mean ± SEM, $n = 4$) following intraduodenal infusion of formulations from 0 to 2 h. Formulations contained 200 μg Halofantrine (Hf) dispersed in either; 4 mg oleic acid (OA) emulsified in 0.2% Tween 80 in normal saline (●); 4 mg OA emulsified in 5 mM bile salt solution (○); or 4 mg OA/10.4 mg lysophosphatidylcholine emulsified in 5 mM bile salt solution (▲).

Table II. Transport of Endogenous Fatty Acid (FA) (μmol) (Comprising Phospholipid Derived FA) in Bile (Mean ± SEM, n = 3) and Transport of Endogenous FA (μmol) (Comprising Triglyceride and Phospholipid Derived FA) in Lymph (n = 4) Over the 3–5 h Period Following Commencement of a 2-h Intraduodenal Infusion of Different Formulations to Anesthetized Rats

Formulation	Biliary transport of endogenous FA (μmol)	Lymph transport of endogenous FA (μmol)
Normal Saline	14.6 ± 0.5	19.4 ± 1.2
4 mg oleic acid (OA) dispersed in 0.2% Tween 80 in normal saline	9.4 ± 0.4	19.4 ± 1.0
4 mg OA dispersed in 5 mM bile salt solution	31.0 ± 0.7[a]	29.3 ± 1.2[a]
4 mg OA/10.4 mg lysophosphatidylcholine dispersed in 5 mM bile salt solution	31.0 ± 0.6[a]	51.6 ± 3.3[a,b]

All oleic acid formulations contained 200 μg halofantrine.
[a] Statistically different to both normal saline and 4 mg OA dispersed in 0.2% Tween 80 groups ($p < 0.05$).
[b] Statistically different to 4 mg OA dispersed in 5 mM bile salt solution ($p < 0.05$).

low-dose exogenous FA containing formulation administered to fasted rats. Previous studies have examined lymphatic drug transport following bile diversion; however, this method complicates determination of the intrinsic ability of bile to enhance lymphatic drug transport because the solubilization of lipids within the intestinal lumen is compromised in the setting of bile diversion that subsequently reduces the efficiency of lipid absorption into the enterocyte (31,32). The current results indicate that endogenous FA in lymph that are recruited from bile more avidly support lymphatic transport of Hf than other sources of endogenous FA. The difference is believed to be due to the disparate intracellular trafficking pathways and pooling of these endogenous FA within the enterocyte.

Endogenous FA Transport in Bile and Intestinal Lymph

The low lipid dose of 4 mg OA (emulsified in 0.2% Tween 80), which is approximately equivalent to administration of a unit dose capsule of a lipid-based formulation to humans (on a mg/kg basis), did not enhance endogenous FA transport in lymph when compared to infusion of normal saline (Table I). This finding was in contrast to our previous studies in canines, where a similarly small amount of long chain lipid (on a mg/kg basis) led to an apparent recruitment of endogenous FA transport into lymph (22). However, increasing the lipid dose administered to the rats to 40 mg OA led to a significant increase in the recruitment of endogenous FA transport in lymph (Table I). This finding is consistent with a previous report in rats, where relatively small amounts of exogenous lipid were shown to recruit endogenous lipid transport into lymph in a dose-dependent manner (7). It is likely that the difference between the current rat data and the previous canine data are a function of formulation differences (the dog study employed a micro-emulsion formulation, whereas the current study employed an emulsion), differences with respect to the mass of lipid required to recruit biliary lipid secretion and endogenous FA transport in lymph in rats and dogs, and interspecies differences in intestinal lipid processing and BS concentrations. In particular, rats (in contrast to dogs) do not have a gallbladder that stores and releases bile after ingestion of lipid, and the rate of bile output is believed to be relatively constant whereas in dogs bile output is expected to increase following a lipid meal.

The addition of BS to the formulation containing 4 mg of OA, significantly enhanced endogenous FA output in bile (Table II) and endogenous FA transport in lymph (Tables I and II). This result suggests that the increase in endogenous FA transport in lymph was at least partially dependent on an increase in endogenous FA output in bile (in the form of PL). To our knowledge, this is the first demonstration of the fact that intestinal administration of a small amount of BS can stimulate a rapid increase in biliary PL output (within a 2- to 3-h period), leading to an increase in endogenous FA transport in lymph. Biliary lipid secretion (PL and cholesterol) is known to be stimulated by the presence of high cannilicular lumen levels of BS (33). The BS added to the formulation may therefore undergo enterohepatic circulation to the hepatic canniliculi, where it stimulates an increase in biliary PL secretion that in turn leads to an increase in endogenous FA transport in lymph.

The addition of LPC to the formulation containing 4 mg OA dispersed in BS solution further enhanced endogenous FA transport in lymph (Fig. 1; Tables I and II). Interestingly, the endogenous FA recruited into lymph by addition of LPC did not originate from enhanced endogenous FA output in bile (Table II). The most likely endogenous FA recruited by the presence of LPC are basolaterally sourced endogenous FA taken up from the intestinal blood supply (7,9), as there is only a small and relatively constant amount of endogenous FA in lymph derived from either sloughed enterocytes or *de novo* synthesis (2,7,34). This suggestion is consistent with previous studies in rats which detailed the source of recruited lymphatic lipids following addition of 9 μmol/h phosphatidylcholine (PC) (of which LPC is the absorbed digestion product) to a continuous, steady-state intraduodenal infusion consisting of 135 μmol/h triolein (TO) emulsified in 10 mM bile salt solution (35,36). These previous studies showed that addition of PC to the TO dispersed in BS solution enhanced the overall transport rate of triglyceride in lymph from 50 to 110 μmol/h. Basolaterally sourced endogenous lipids seemed to be the source of the increase in lymphatic lipid output because an eightfold reduction in the mass of lipid present in a mucosal pool consisting of lipids taken up across the basolateral membrane was observed. Overall, PC addition seemed to redirect lipids from this pool for transport to the systemic circulation via the intestinal lymph.

In the current studies, a similar attempt was made to determine whether the endogenous FA in lymph recruited by the presence of LPC were basolaterally sourced. In these experiments, the duodenum, mesenteric lymph duct, and jugular vein of rats were cannulated. ^{14}C-OA was continuously infused into the jugular vein to provide a steady-state blood concentration of ^{14}C-OA as a marker for endogenous FA in the blood, and potentially to provide a source of

radiolabeled endogenous lipid for uptake from the blood across the basolateral membrane of the enterocyte. After the attainment of steady-state blood concentrations of ^{14}C-OA, the formulation containing 4 mg OA emulsified in BS was then infused intraduodenally in the presence, or absence, of LPC, and the transport of ^{14}C-OA (as a marker for basolaterally sourced endogenous FA) into the lymph assessed. However, during the course of the validation experiments it became evident that substantial amounts of the intravenously infused ^{14}C-OA were recovered in bile, precluding the use of ^{14}C-OA as a specific marker for the basolateral uptake of endogenous FA from the intestinal blood by enterocytes (unpublished data).

Lymphatic Drug Transport

Endogenous lymphatic FA (Fig. 1 and Table I) and drug transport (Fig. 2) were both stimulated by addition of BS to the intraduodenal infusion of 4 mg OA. In contrast, addition of LPC to the infusion containing 4 mg OA dispersed in BS solution did not change lymphatic drug transport (Fig. 2) in spite of the significantly enhanced endogenous FA transport in lymph (Fig. 1 and Table I). This observation was surprising as lymphatic drug transport is generally considered to be dependent on lymphatic lipid transport (22). An explanation as to why addition of BS to the 4-mg OA formulation increased lymphatic drug transport, whereas addition of LPC did not, despite both BS and LPC stimulating endogenous FA transport in lymph, may be related to the source of recruited endogenous FA in lymph. For example, addition of BS stimulated an increase in endogenous FA output in bile and endogenous FA transport in lymph (Table II), and this was reflected in an increase in lymphatic drug transport (Fig. 2). In contrast, despite addition of LPC leading to a substantial increase in lymphatic endogenous FA transport (Table II), it did not stimulate an increase in biliary endogenous FA transport (Table II) nor was there any enhancement in lymphatic drug transport (Fig. 2).

This result strongly suggests that biliary-derived endogenous FA, which are absorbed from the intestinal lumen into the enterocyte and subsequently transported to the systemic circulation via the intestinal lymphatic system, are able to support substantial fasted state lymphatic transport of Hf. In contrast, endogenous FA of nonbiliary origin (such as those sourced from the intestinal blood via the basolateral membrane of the enterocyte) and stimulated to enter the lymph by addition of LPC to the formulation do not seem able to support a coincident increase in lymphatic transport of Hf. The results indicate that each source of endogenous FA forms a separate pool or repository of intracellular lipids that differ in their propensity to coincidentally enhance lymphatic lipid and Hf transport.

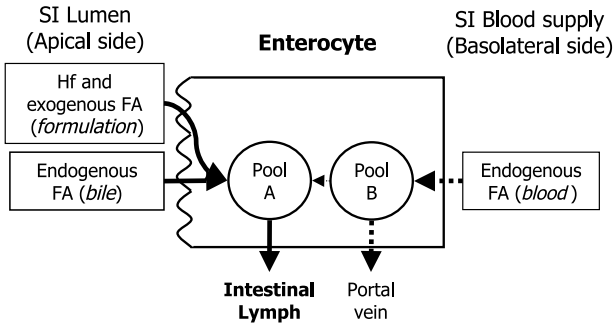

Fig. 3. Schematic representation of the sources and pools of recruited endogenous FA in lymph that support lymphatic lipid and halofantrine (Hf) transport.

Two separate pools of lipid have been isolated from the intestinal mucosa, and these have been well described in the biology literature (9,35–39). One pool (the lymph lipid precursor pool or pool A) is located in the SER and golgi, has a lipid content reflective of the diet, and subserves CM formation; moreover, lipid from this pool is predominantly transported to the systemic circulation via the mesenteric lymph (9,35–39). The data presented here suggest that lipids in this pool are most readily able to support lymphatic Hf transport. The second pool of lipids (pool B or the portal lipid precursor pool) is located within the cytosol and is predominantly composed of lipids entering the enterocyte via basolateral uptake from the intestinal blood. The lipids from pool B are generally transported from the enterocyte to the systemic circulation via the portal vein (although a portion of the lipid in this pool may be transported into the mesenteric lymph via transfer to pool A) (9,35–39). The data described here suggest that this pool of endogenous lipids does not support lymphatic transport of Hf.

Figure 3 is a schematic representation of the sources and pools of recruited endogenous lipid that support lymphatic lipid and Hf transport consistent with the data and findings of this study. Bile containing endogenous FA (in the form of PL) is secreted into the SI lumen, and this endogenous FA is absorbed across the apical membrane and incorporated into the lymph lipid precursor pool (pool A) in the enterocyte. The intestinal blood supply provides endogenous FA that are taken up across the basolateral membrane and predominantly incorporated into the portal lipid precursor pool (pool B) in the enterocyte. The lipid from pool A is mostly transported to the systemic circulation via the intestinal lymphatic system and the lipid from pool B is mostly transported via the portal vein. A proportion of the lipid in pool B may, however, transfer to pool A for eventual transport to the systemic circulation via the intestinal lymphatic system. When an exogenous lipid–drug formulation is administered, endogenous FA may be recruited for lymph transport from either the bile or the intestinal blood supply. Endogenous FA in lymph sourced from bile support drug association with the lipid in pool A, and subsequently drug transport via the lymphatic system. In contrast, endogenous FA in lymph sourced from the intestinal blood supply that enter pool A via pool B are unable to support Hf association with the lipid in pool A or lymphatic drug transport.

CONCLUSION

The current study was undertaken to elucidate whether different sources of recruited endogenous FA (biliary or blood derived) in lymph, and bile components in general, are able to support Hf transport in lymph. Not all sources of endogenous FA in lymph were equivalent with respect to their ability to support Hf transport in lymph. Endogenous

FA derived from bile most readily supported lymphatic transport of Hf, whereas an alternate source of endogenous FA (most likely derived from basolateral uptake of lipids from the intestinal blood supply) did not support Hf transport in lymph. As endogenous FA sourced from either the luminal or basolateral side of the enterocyte enter different mucosal lipid pools, it is hypothesized that the pool of lipid (pool A) that bile-derived endogenous FA enter is able to support lymphatic transport of Hf, whereas the other lipid pool (pool B) does not. This finding suggests that the different mucosal lipid pools can directly influence lymphatic transport of Hf. Future studies will examine the impact of changes to the size and dynamics of the lymph lipid precursor pool (pool A) to lymphatic drug transport.

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