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Lipid-based delivery systems and intestinal lymphatic drug transport: A mechanistic update

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Received 1 September 2007; accepted 30 September 2007

Available online 7 November 2007

Abstract

After oral administration, the majority of drug molecules are absorbed across the small intestine and enter the systemic circulation via the portal vein and the liver. For some highly lipophilic drugs (typically log \( P^N > 5 \), lipid solubility > 50 mg/g), however, association with lymph lipoproteins in the enterocyte leads to transport to the systemic circulation via the intestinal lymph. The attendant delivery benefits associated with lymphatic drug transport include a reduction in first-pass metabolism and lymphatic exposure to drug concentrations orders of magnitude higher than that attained in systemic blood. In the current review we briefly describe the mechanisms by which drug molecules access the lymph and the formulation strategies that may be utilised to enhance lymphatic drug transport. Specific focus is directed toward recent advances in understanding regarding the impact of lipid source (both endogenous and exogenous) and intracellular lipid trafficking pathways on lymphatic drug transport and enterocyte-based first-pass metabolism.

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Keywords: Drug; Lymph; Absorption; Intestine; Formulation; Lipid; Delivery; Oral

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This review is part of the Advanced Drug Delivery Reviews theme issue on “Lipid-Based Systems for the Enhanced Delivery of Poorly Water Soluble Drugs”.

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doi:10.1016/j.addr.2007.09.007
1. Introduction

It is becoming evident that pressure to identify increasingly potent lead compounds is leading to the identification of increasingly lipophilic drug candidates [1]. In response, and as described throughout this theme issue, a growing number of formulation technologies have been developed to support the absorption of compounds with very low water solubility and high lipophilicity. In combination, these factors have led to more common, and more confident, pre-clinical and clinical progression of compounds with physicochemical characteristics that are consistent with the potential for lymphatic transport (typically log \( P > 5 \), long chain triglyceride (TG) solubility \( > 50 \, \text{mg/g} \)) [2]. As such an understanding of the mechanisms of drug access to the intestinal lymph, the potential ramifications of drug transport to the systemic circulation via the intestinal lymph rather than the portal blood and the impact of formulation changes on intestinal lymphatic transport is of significance to a much broader audience.

The area of intestinal lymphatic transport has been reviewed by ourselves and others in recent years [3–6] and the aim of the current article is therefore not to revisit in detail many of the general aspects that have been well covered previously. Rather we focus here on recent advances in our understanding of the mechanisms of drug access to the intestinal lymph and the importance of enterocyte-based lipid processing in determining patterns of lymphatic drug transport.

2. Overview of intestinal lymphatic drug transport

2.1. General aspects of drug access to the intestinal lymph

The basic principles that dictate the mechanism by which drugs enter the intestinal lymph following oral delivery are summarised in Fig. 1. The gastrointestinal tract is richly supplied with both lymphatic and blood vessels and therefore materials that are absorbed across the small intestinal epithelial cells (enterocytes) can potentially enter either lymphatic or blood capillaries. The majority of absorbed materials are transported into the portal blood because the rate of fluid flow in the portal blood is approximately 500-fold higher than that of the intestinal lymph. However, where facile diffusion across the blood capillary endothelium is limited, for example for high molecular weight or colloidal materials, selective transport into the intestinal lymph may occur since the endothelial architecture of the lymphatics dictates that lymphatic capillaries are significantly more permeable than the neighbouring blood capillaries [7]. Absorption of macromolecular drug constructs into and across the enterocyte however, is limited, and drug access to the intestinal lymph more commonly occurs as a result of post-absorptive association with colloidal lipoproteins during transport across the enterocyte (see Fig. 1). The physical size of the lipoproteins subsequently dictates that diffusion across the vascular endothelium is limited and that preferential access of lipoproteins (and associated drug) to the lymphatics occurs. This mechanism is supported by studies demonstrating that the majority of lymphatically transported DDT [8], aryl and alkyl hydrocarbons [9] and halofantrine [10] are solubilised within the apolar lipid core of lymph lipoproteins.

Lymphatic transport has been shown to be a contributor to the oral bioavailability of a number of highly lipophilic drugs and other xenobiotics following oral delivery, including: two lipophilic cannabinoids [184], halofantrine [11,12], moxidectin [13], mepitiostane [14,15], testosterone derivatives [16], MK-386 (a 5α-reductase inhibitor) [17], peniclomedine [18], naffitine [19], probucol [20], cyclosporine [21], ontazolast [22], CI-976 [23], fat soluble vitamins and their derivatives, retinoids [24], lycopene, DDT and analogs [8,25], benzopyrene, PCBs (polychlorinated biphenyls) [26] and a number of lipophilic prodrugs [27–29]. In contrast, only very small quantities of more hydrophilic drugs such as salicylic acid, isoniazid and caffeine are recovered in lymph following oral delivery [8].
2.2. Strategies to enhance lymphatic drug transport

Realisation that drug access to the intestinal lymph is dependent on drug association with developing lipoproteins in the enterocyte suggests that the provision of an appropriate lipid source to drive lipoprotein assembly is a key strategy to enhance lymphatic drug transport. Indeed the efficiency of lipid digestion and solubilisation in the intestinal lumen (reviewed elsewhere in this issue and previously [5,6,30]) and subsequent uptake and transport across intestinal absorptive cells is likely to significantly influence the access of lipophilic drugs to the lymph. Both the type and mass of co-administered lipid can alter the extent of lymphatic drug transport and this subject has been well reviewed previously [3–5,31]. Briefly, fatty acids (FA) with chain lengths of 14 or greater are more highly lymphatically transported (~40–60% of the lipid dose is transported to the systemic circulation via the intestinal lymph with the remainder absorbed via the portal vein blood [32–34]), whereas shorter chain FA, which are more water soluble, are primarily absorbed via the portal blood [35–37]. As such, long chain FA and triglycerides (TG) composed of long chain FA more effectively support lymphatic drug transport than their medium and short chain counterparts [20,24,38–46]. The degree of unsaturation of administered FA also influences the extent of lymphatic lipid and drug transport. In general, mono- and poly-unsaturated FA (MUFA and PUFA, respectively) promote lymphatic lipid transport more readily, produce larger sized lipoproteins and therefore enhance lymphatic drug transport more effectively when compared with the equivalent saturated FA [41–47]. Phospholipids (PL) and in particular phosphatidylcholine (PC) and its digestion product, lyso-phosphatidylcholine (LPC) also enhance lymphatic lipid transport and LPC has been shown to enhance the lymphatic transport of α-tocopherol [48] and more recently, halofantrine [49].

A prodrug approach may also be taken to enhance lymphatic drug transport via the covalent coupling of drugs to lipid moieties including fatty acid, diglyceride or phosphoglyceride [28]. Simple fatty acid esters esterify to enhance lipophilicity and therefore drug association with lipoproteins in the enterocyte, however the instability of the ester linkage in the intestine and enterocyte typically results in relatively inefficient lymphatic targeting. Glyceride or phospholipid-based prodrugs, however, are designed to mimic, and intercalate into, the glyceride or phospholipid resynthetic pathways within the enterocyte and are typically more stable and therefore lead to enhanced lymphatic recovery. Lymph-directing prodrug strategies have been reviewed in detail elsewhere [27–29].

2.3. Advantages of intestinal lymphatic drug transport

Due to the unique anatomy and physiology of the lymphatics, intestinal lymphatic drug transport can provide a number of advantages over drug absorption via the portal blood. For example, drugs which are absorbed via the intestinal lymphatic system are essentially protected from hepatic first-pass metabolism since the mesenteric lymph, in contrast to the portal blood, enters the systemic circulation directly without first passing through the liver. For drugs which are highly metabolised on first pass through the liver, transport via the lymphatic system can therefore significantly enhance oral bioavailability. The lymphatic system is also the principle systemic transport pathway for B and T lymphocytes as well as the primary route of metastatic spread of a number of solid tumours [50,51]. As such it has been suggested that immunomodulatory and anticancer compounds may be more effective when absorbed via the lymphatic route [52,53]. Recent evidence further suggests that lymph and lymphoid tissue, and in particular gut associated lymphoid tissue, play a major role in the development of human immunodeficiency virus (HIV) [54,55] and antivirals which target AIDS may therefore be more effective when absorbed via the intestinal lymphatics. Indeed, a recent communication describes the synthesis of lipidic prodrugs of didanosine designed to improve the treatment of HIV [56]. Other viruses may also spread via the lymphatic network including hepatitis B [57], morbillivirus [58] (which also replicates in gut associated lymphoid tissue) and the closely related canine distemper virus [59], severe acute respiratory syndrome (SARS) associated coronavirus [60] and the chronic persistence of hepatitis C is believed to result from uptake into systemic lymphocytes and sequestration into the lymph [61]. Conversely, drug transport via the intestinal lymph results in an altered pattern of local drug exposure to the lymphatics and a changed mode of delivery to the systemic circulation, and therefore potential changes to toxicological profiles are also possible.

3. Recent advances in the understanding of lymphatic drug transport

3.1. Research models

3.1.1. In vivo models

Evaluation of intestinal lymphatic drug transport requires invasive and largely irreversible surgery to access and cannulate the intestinal lymphatic duct. As such, lymphatic drug transport cannot be studied directly in humans. Various animal models have therefore been described in an attempt to quantitate the contribution of the lymphatic system to overall drug absorption [62]. In the majority of cases these pre-clinical models collect the entire volume of lymph flowing through mesenteric or thoracic lymph duct cannulas and therefore provide an absolute indication of the extent of lymphatic transport. Other models have also examined the use of a lympho-venous shunt which has the advantage of allowing sampling of lymph over much longer periods, although in this case the relatively small database for lymph flow rates makes estimation of the absolute extent of lymphatic transport difficult [63]. The majority of lymphatic transport studies described in the literature have utilised rats [62], reflecting the relative ease of sourcing and housing small laboratory animals, however, larger animal models such as dogs [11–13], pigs [64], sheep [65,66] and rabbits [67] have also been described. The advantages of larger animal models include the capacity to dose more clinically relevant full-sized human dosage forms and the ability to administer compounds under more representative fed and fasted conditions.
states (rodents do not eat on command). The gastrointestinal tract, transit profile and biliary secretion patterns of dogs and pigs are also more similar to that of humans when compared with rats (in which bile is continuously secreted into the intestine). However, the complexity and cost of larger animal models typically limits widespread application.

Recently, an alternate in vivo approach to the estimation of intestinal lymphatic drug transport has been described in which the systemic exposure of drug is assessed after drug administration in the presence and absence of an inhibitor of intestinal chylomicron flow (e.g. Pluronic-L81 or colchicine) [68]. Comparison of systemic drug exposure profiles in the presence and absence of a functional intestinal lymphatic system provides an indication of the importance (or otherwise) of lymphatic drug transport to overall bioavailability. This approach has the advantage of not requiring the surgical interventions inherent in lymph duct cannulation, however, the broader implications of blocking chylomicron flow and intestinal lipid processing on drug exposure (and indirectly, lymphatic transport) are yet to be studied in detail.

3.1.2. In vitro models

The use of in vitro models as an alternate to in vivo models of assessment of lymphatic drug transport has also been described. For example, Caco-2 cells are well recognised in the pharmaceutical arena as an in vitro model of intestinal epithelium and are widely utilised to screen for intestinal permeability properties. However, Caco-2 cells have also been employed in the lipid biochemistry literature to examine aspects of intracellular lipoprotein assembly [69] and have recently been evaluated as a prospective in vitro model to examine the influence of lipids and lipidic excipients on drug incorporation into lipoproteins and lymphatic transport [70–72]. These data are reviewed in more detail elsewhere in this theme issue [73].

Gershkovich and Hoffman [74] have also suggested that the degree of ex vivo association of drugs with chylomicrons harvested from plasma may be used as a simple predictive tool as to the likely extent of lymphatic drug transport. In these studies, a reasonable linear correlation ($r^2 = 0.94$) was obtained between the extent of lymphatic transport of several lipophilic drugs and their degree of association with plasma chylomicrons ex vivo. Importantly, this correlation was substantially better than that between the extent of lymphatic drug transport and TG solubility or log $P$. An in silico method aimed at developing a quantitative relationship between molecular structure and the extent of intestinal lymphatic drug transfer has also recently been described [75]. The authors found that a relatively complex set of molecular descriptors was required to predict the likelihood of lymphatic transport, although once again the approach appeared to give more accurate predictions than that obtained using traditional descriptors (such as log $P$ and TG solubility).

3.2. Lipid transport processes and drug absorption

3.2.1. Lipid transporters and binding proteins

A number of lipid transport proteins have been identified on both the apical and basolateral membranes of enterocytes which, together with several families of intracellular lipid binding proteins, facilitate the absorption and intracellular transport of endogenous and dietary lipids. These transporters and binding proteins have been reviewed in detail elsewhere [76, 77]. Fig. 2 depicts a schematic which summarises the lipid transporters and binding proteins that have been implicated in the uptake and transport of various lipids across intestinal epithelial cells. Briefly, lipid uptake across the apical membrane of enterocytes may proceed by either active transport [78, 79] or passive diffusion [80] and a number of transporters have been implicated in apical membrane uptake of FA (e.g. CD36/FAT [81, 82], scavenger receptor BI (SR-BI) [83, 84] and FABP$_{\text{pm}}$ [85]) and cholesterol [86] (e.g. SR-BI [87, 88], caveolin [89], CD36/FAT [90], aminopeptidase N [86, 91], caveolin-1/annexin

![Fig. 2. Lipid transporters and binding proteins involved in intestinal lipid absorption. A number of transporters and binding proteins have been implicated in lipid uptake into and transport across intestinal epithelial cells (enterocytes) although the relative contribution of each of these proteins and indeed the role of passive vs active uptake in the overall intestinal absorption of lipids is yet to be fully resolved. Apical membrane lipid transporters (including CD36/FAT (cluster determinant 36/fatty acid translocase), FABP$_{\text{pm}}$ (plasma membrane fatty acid binding protein), SR-BI (scavenger receptor BI), caveolin-1 (which may act in concert with annexin-2), aminopeptidase N, NPC1L1 (Niemann Pick C1-Like 1), (dark circles)) are believed to play a role in the uptake of lipid digestion products including fatty acid (FA), cholesterol (Ch) and other sterols, monoglycerides (MG) and lyso-phosphatidylcholine (LPC) from the intestinal lumen into the enterocytes. Lipid digestion products may also be effluxed from enterocytes back into the intestinal lumen by ABC (ATP-binding cassette) efflux transporters (black triangles). Transport across the enterocyte cytoplasm is thought to be facilitated by intracellular lipid binding proteins (including I-FABP (intestinal fatty acid binding protein), L-FABP (liver fatty acid binding protein), SCP (sterol carrier protein) (dark rings)). Ch exit from enterocytes across the basolateral membrane is facilitated by ABCA1 and a number of as yet unidentified transporters may facilitate exit of other lipids across the basolateral membrane (white circles).]
2 complex (the role of which is less clear) [86] and NPC1L1 [92,93]). The relative importance of each of these transporters to overall transport, however, is yet to be distinguished. Protein mediated transfer across the apical membrane of enterocytes has also been demonstrated for lyso-phospholipids and phospholipids [94], cholesterol ester [95] and monoglyceride [96–98] although the transporters involved are less well defined that those for FA and cholesterol.

Several ATP-binding cassette (ABC) transporters have been implicated in lipid uptake across plasma membranes and intracellular lipid trafficking at sites other than the small intestine [99–102]. ABC transporters may therefore be involved in intestinal lipid absorption, although currently, the role of only a few of these transporters has been demonstrated. For example P-glycoprotein is believed to influence intestinal lipoprotein formation [70,101,103] and it has been suggested that P-glycoprotein facilitates the absorption and intracellular trafficking of cholesterol although the evidence for this is still circumstantial [86]. Additionally, ABCA1 appears to facilitate exsorption of cholesterol across the basolateral membrane of enterocytes to plasma ApoA-1 which enhances the formation of nascent HDL [86,102,104] and ABCG5 and ABCG8 are thought to reduce excess intestinal cholesterol and sterol absorption by facilitating efflux from enterocytes [86,102,105].

More recently, evidence has also been documented of the intact transfer of macromolecular lipid complexes (such as lipoproteins or fatty acids bound to albumin) across the plasma membrane of hepatocytes, adipocytes and endothelial cell lines by incorporation into vesicles derived from the plasma membrane via clathrin- or caveolae-mediated endocytosis [106–108]. As such, endocytosis may play a role in the uptake of dietary lipid from the small intestine in the form of mixed micellar or vesicular species.

Following absorption, lipid digestion products such as FA, Ch, MG and LPC appear to cross the enterocyte cytoplasm by passive diffusion. During cytoplasmic diffusion, intracellular solubilisation of lipids is facilitated by association with intracellular lipid binding proteins (ILBPs) including intestine and liver fatty acid binding proteins (I-FABP and L-FABP respectively) [77,109,110], sterol carrier protein (SCP) [111,112], retinol and retinoic acid binding proteins [113] and ileal bile acid binding protein (I-BABP) [110].

3.2.2. Lipid transporters, binding proteins and lymphatic drug transport

There is increasing, albeit limited, evidence that both membrane-resident lipid transporters and intracellular lipid binding proteins (ILBPs) may impact on the uptake and intracellular disposition and trafficking of drug molecules. This may occur either indirectly via changes to the patterns of intracellular trafficking and disposition of lipids, which in turn alter patterns of drug association with intracellular lipid pools; or via direct drug interaction with lipid transporters or ILBPs.

For example, structural studies have demonstrated that I-FABP and L-FABP bind with relatively high affinity to certain drugs, and in particular those with structural similarities to the endogenous ligand FA [114,115]. Drug binding to I-FABP has in addition been shown to enhance the transport of lipophilic drug molecules across a model artificial membrane, where the degree of transport enhancement was related to both drug lipophilicity and I-FABP binding affinity (100). A recent study has also demonstrated a correlation between the level of expression of I-FABP and L-FABP mRNA in small intestinal epithelial cells and the rate of transport of lipid and a model drug (halofantrine) into the intestinal lymph [116]. These results suggest that I-FABP and L-FABP may influence lymphatic drug transport although further data is required to confirm a causal relationship between FABP levels and lymphatic drug transport. It is also unclear whether FABP influences lymphatic drug transport directly by binding to and facilitating drug transport or indirectly by facilitating lymphatic lipid transport. Interestingly, L-FABP and to a lesser extent, I-FABP were recently shown to initiate the ER budding of a pre-chylomicron vesicle which transports lipids from the ER to the Golgi and is the primary rate limiting step of lipid transport into the lymph [117]. L-FABP and I-FABP may therefore have a broader and more influential role in lymphatic lipid transport than previously suspected.

The levels of I-FABP and L-FABP mRNA may be up-regulated acutely by administration of relatively small quantities of lipid over a time course (2–5 h) of potential relevance to the absorption of lipid-based drug formulations, particularly under multiple or chronic dosing situations [116]. These acute data are consistent with previous studies which have demonstrated transcriptional up-regulation of I-FABP and L-FABP following chronic ingestion of high fat diets by both rats and mice [118–120]. Interestingly, the regulatory proteins (e.g. peroxisome proliferator activated receptors (PPAR) [118,121]) which influence FABP transcription and expression in response to lipid ingestion further influence the transcriptional expression of a number of proteins implicated in intestinal lipid absorption [122]. FABP may therefore be only one of a number of co-ordinately regulated proteins which are involved in an acute intestinal response to lipid ingestion and which in turn influence the rate and extent of intestinal lymphatic lipid and drug transport. Clearly, significantly more data is required to confirm (or refute) these suggestions.

3.3. Triglyceride resynthesis and lipoprotein assembly

The pathways which dictate the intestinal uptake and resynthesis of lipid digestion products and subsequent access of lipids to the systemic circulation via either the intestinal lymphatic system or portal vein blood are described in Fig. 3. Essentially, following uptake into the enterocyte, lipid digestion products either diffuse across the cell and enter the portal vein capillaries directly, or are trafficked to the endoplasmic reticulum (ER) where they are resynthesised to TG, PL or CE. TG resynthesis occurs via 2 pathways; the 2-monoglyceride (2-MG) pathway (located in the smooth endoplasmic reticulum (SER)) or the glycerol-3 phosphate (G3P) pathway (located in the rough endoplasmic reticulum (RER)) [30]. In comparison, LPC is resynthesised to PC [123–125] by lyso-phospholipid: Acyl-CoA acyltransferase in the SER and a proportion of the LPC is also hydrolysed to form glycerol-3-phosphorylcholine
Fig. 3. Intracellular processing of lipids in the enterocyte. Following uptake across the apical membrane of the enterocyte, the products of gastrointestinal (GI) lumen lipid digestion (e.g. monoglyceride (MG) and fatty acid (FA)) may either diffuse across the enterocyte and enter the portal vein blood [34] or be resynthesised to triglyceride via either the 2-monoglyceride (2-MG) pathway associated with the smooth endoplasmic reticulum (SER) or the glycerol-3-phosphate (G3P) pathway associated with the rough endoplasmic reticulum (RER) [134,138]. Triglyceride formed via these pathways may enter the endoplasmic reticulum lumen where the triglyceride is assembled into lipoproteins (LP, represented by circles). LP are then transported to the Golgi, exocytosed from the enterocyte and taken up into the intestinal lymphatic system [6]. Since lipid contained within the lipoprotein assembly pathways and the Golgi is destined for transport to the systemic circulation via the intestinal lymphatic system, this pool of lipids is referred to as the lymph lipid precursor pool (dashed blue line) [49,140]. A cytosolic pool of lipids is also located within the enterocytes [49,140]. This lipid pool comprises excess triglyceride formed via the G3P pathway [135] and endogenous lipids taken up from the intestinal blood supply in the form of either FA or chylomicron remnants [137,141]. These cytosolic lipids are subject to hydrolysis by cytosolic lipase [140] and the digestion products so formed may be re-circulated into TG assembly pathways [135]. However, the majority of lipids from this pool exit the enterocyte in the form of TG or free FA and are taken up into portal vein blood [49,140]. The pool of lipids which is transported from the enterocyte via the portal vein is therefore referred to as the portal lipid precursor pool (dashed red line) [49,140]. This figure is modified from reference [5].

which is transported via the portal vein to the liver [126,127]. Ch absorbed from the luminal side of the enterocyte, particularly in the fed state, is esterified to CE by acyl-coA: cholesterol-O-acyltransferase 2 (ACAT2) [128–130] and preferentially incorporated into lipoproteins for absorption into the intestinal lymph [42,131,132]. In the fasted state very little Ch is transported into the lymph [131] and most enters a free Ch pool within the enterocyte [133].

The contribution of the 2-MG and G3P pathways to intestinal TG resynthesis is in large part dictated by the sources of lipid present. Thus, the 2-MG pathway is the primary pathway for TG synthesis when exogenous 2-MG is available e.g. following ingestion of TG, diglyceride (DG) or 2-MG [134–136]. In contrast, in the absence of exogenous glycerides (e.g. in the fasted state or following administration of FA, rather than glycerides), the G3P pathway is the major pathway of TG synthesis [134–136]. The major portion of TG formed via the 2-MG pathway crosses the SER membrane and enters lipoprotein assembly pathways [135,136]. Lipids which enter lipoprotein assembly pathways within the enterocyte (such as those resident within the ER and Golgi) and are destined for transport from the enterocyte via the intestinal lymph have been referred to as residing in the lymph lipid precursor pool [49] or chylomicron precursor pool [137]. Since the majority of lipids which enter the lymph lipid precursor pool are formed via the 2-MG pathway, the composition of the lymph lipid precursor pool consists primarily of endogenous lipids in the fasted state but increasingly reflects that of exogenous lipids following ingestion of lipids [49,138].

In addition to the TG formed via the 2-MG pathway, a (relatively minor) fraction of the TG produced via the G3P pathway is also incorporated into lipoproteins [139]. However, the majority of TG synthesised via the G3P pathway enters a pool of lipid droplets which is diffusely distributed throughout the enterocyte cytoplasm. This cytosolic pool of lipids has been referred to as the ‘portal lipid precursor pool’ since the majority of lipids in this pool are transported to the systemic circulation via the portal vein [140]. Endogenous fatty acids and CM remnants from the intestinal blood supply which are taken up into the enterocytes across the basolateral membrane also enter the portal lipid precursor pool [137,141]. As such, the portal lipid precursor pool consists primarily of basolaterally-sourced endogenous lipids from the intestinal blood supply [137,141] and also endogenous lipids synthesised via the G3P pathway. Since the portal lipid precursor pool is located in the cytoplasm and not surrounded by a membrane, the lipids in the portal lipid precursor pool may be hydrolysed [134,136] by cytosolic lipase [142]. Once hydrolysed, the lipids may be transported to the portal vein or resynthesised via the 2-MG pathway and also incorporated into lipoprotein assembly pathways and the lymph lipid precursor pool [140].

Aspects of lipoprotein assembly occur within both the SER and RER of enterocytes. The first step in the sequential assembly
of lipoproteins involves formation of a primordial lipoprotein within the RER. This is initiated by the co-translational integration of ApoB48 (an apo-protein found on the surface of intestinal lipoproteins and which is believed to be essential for the formation of intestinal lipoproteins) into the RER membrane [143,144] followed by facilitated association of phospholipid with ApoB48 which is mediated by microsomal triglyceride transport protein (MTP) [145,146]. The ‘lipidated’ ApoB48 (or primordial lipoprotein) is then released into the RER lumen [139]. The second step in the sequential assembly of lipoproteins involves formation of TG droplets within the SER. TG synthesised on the surface of the SER via the 2-MG pathway is believed to enter the SER membrane via a process facilitated by MTP [147,148]. Saturation of the SER membrane by TG occurs relatively rapidly and the TG forms a small lens within the SER membrane which eventually pinches off from the membrane and forms a TG droplet on the luminal side of the SER [149]. The size of the TG droplets formed within the SER and thus the subsequent size of the assembled lipoproteins is believed to depend on the mass and type of administered lipid. The final step of lipoprotein assembly involves fusion of the TG droplets (SER derived) with primordial lipoproteins (RER derived) [139] at the junction of the SER and RER leading to the formation of a nascent lipoprotein. The nascent lipoprotein is subsequently transported in association with a ‘pre-chylomicron transport vesicle’ (PCTV) to the Golgi apparatus [150] and from the Golgi to the basolateral membrane in Golgi derived vesicles which contain multiple lipoproteins [151]. These vesicles fuse with the basolateral membrane of the enterocytes and the lipoproteins are discharged into the intercellular space underlying the enterocyte from where they are free to diffuse through the connective tissue of the lamina propria to the lymphatic capillaries.

The size, density and therefore types of lipoproteins (e.g. very low density lipoproteins (VLDL) or chylomicrons (CM)) formed in the intestine are thus largely dependent on the mass and type of lipid ingested. In the fasted state or following administration of PL [152], the TG droplets formed within the lymph lipid precursor pool are relatively small leading to the formation of VLDL (diameter 60–80 nm, sedimentation coefficient \(S_v\) 20–60, 0.93 < \(\rho\) (density) < 1.006 g/mL) [139,152] which comprise primarily of endogenous lipid resynthesised to triglyceride via the G3P pathway. In contrast, in the postprandial state or after administration of exogenous lipid, the TG droplets formed within the lymph lipid precursor pool are larger leading to the progressive formation and secretion of relatively large and exogenous lipid enriched CM [138,153] (diameter 75–400 nm, \(S_v\) ≥ 60, \(\rho\) < 0.93 g/mL), in addition to VLDL, into the lymph [43,154,155].

3.4. Endogenous lipids and lymphatic drug transport

3.4.1. Biological sources of endogenous lipids

Whilst lymphatic lipid transport and lipoprotein formation increases substantially following a fatty meal, even in the fasted state, endogenous lipid flux from the intestine to the mesenteric lymph is maintained [156–158]. For example, intestinal VLDL assembled from endogenous lipid sources contribute approximately 11–40% of total fasted plasma TG [137]. These endogenous lipids may enter the enterocyte across the apical (luminal) or basolateral membranes [141]. Apically-sourced endogenous lipids include those in bile and from desquamated enterocytes. Basolaterally-sourced endogenous lipids include fatty acid and CM remnants taken up from the intestinal blood supply [159–161]. CM remnant uptake is mediated by ApoB100 and ApoE receptors on the basolateral membrane [156]. Endogenous lipids may also be synthesised de novo in the enterocyte.

Of these potential sources of endogenous lipid, apically-sourced, biliary derived lipids are the major contributor to lymphatic lipid transport in the fasted state (~50% in rats) and bile diversion substantially reduces fasted lymphatic lipid transport [137]. Cell desquamation provides a minor source of apically-derived materials [157] and the contribution of de novo lipid synthesis in the enterocyte is thought to be even lower [157]. Basolaterally-sourced endogenous lipids from the intestinal blood are thought to predominantly supply the cytosolic portal lipid precursor pool [157], although portal lipid precursor pool lipids may be redirected to the lymph lipid precursor pool via hydrolysis and resynthesis.

Bile-derived PL is also thought to be required for the formation of lipid-rich (CM) lipoproteins [159–164] and appears to enhance both endogenous and exogenous lipid transport into lymph [165]. The ability of PL to enhance lymphatic transport has been elegantly demonstrated in studies using Mdr2 (−/−) mice where biliary phosphatidylethanolamine secretion is depleted (but normal biliary BS secretion remains). In these animals, postprandial formation of CM does not occur [8,14,166].

3.4.2. Endogenous lipids support lymphatic drug transport

Historically a large mass of exogenous lipid was assumed to be required to stimulate sufficient lymphatic lipid flux and lipoprotein formation to support appreciable lymphatic drug transport. Khoo et al., however, have shown in greyhound dogs that significant fasted state lymphatic drug transport is possible following drug administration with a single unit capsule containing a long chain (LC) lipid-based formulation [12]. The extent of lymphatic drug transport (28.3% of the dose of a model highly lipophilic antimalarial, halofantrine (Hf)) supported by a single unit capsule of formulated LC lipid was substantially greater than the 1.3% recovered in lymph after administration of a lipid free formulation of Hf in the fasted state but was less than the 54% recovered in lymph when dosed following a lipid meal [11]. Interestingly, following administration of the LC lipid formulation, the mass of TG recovered in the lymph (3.4 g over 10 h) was greater than the combination of endogenous TG transport in the lymph in the fasted state (0.5 g over 10 h) and the mass of exogenous lipid dosed (0.58 g), suggesting that administration of the LC lipid formulation led to recruitment of endogenous lipid transport into the lymph. The relatively high extent of lymphatic drug transport therefore appeared to be supported by recruitment of endogenous lymph lipids. The stimulation of endogenous lymphatic transport was
consistent with previous studies in rats where continuous infusion of increasing quantities (from 2.59 mg/h to 26.1 mg/h) of oleic acid over a period of 24 h was shown to increase both endogenous and exogenous lipid transport into intestinal lymph in a dose dependent manner [156,157].

More recently, experiments in lymph-cannulated and bile-duct cannulated rats have further examined the role of endogenous fatty acid recruitment in lymphatic drug transport [167]. In these studies, rats were administered a series of lipid formulations containing halofantrine (Hf), as a model drug, and either 4 mg or 40 mg of oleic acid (OA) over 2 h. The effect of addition of 5 mM bile salt (BS, sodium taurocholate) and lyso-phosphatidylcholine (LPC) was also examined. Administration of 40 mg OA increased both endogenous and exogenous lipid transport into the lymph when compared with administration of either normal saline or 4 mg OA and subsequently enhanced lymphatic drug transport. In contrast, administration of the smaller lipid dose (4 mg of OA) did not stimulate endogenous lymphatic lipid transport above baseline suggesting that endogenous lipid recruitment was (lipid) dose dependent.

Interestingly, addition of 5 mM BS to the 4 mg OA formulation in rats did enhance endogenous lymphatic lipid output and lymphatic transport of Hf (from 7 to 15% of the dose), and in parallel stimulated an increase in biliary lipid secretion. The data therefore suggest that BS infusion stimulates biliary lipid secretion which in turn supports enhanced lymphatic drug transport. In contrast, whilst addition of LPC to 4 mg OA dispersed in BS solution substantially enhanced endogenous lipid transport into lymph, biliary lipid output and lymphatic drug transport did not increase above that obtained after administration of the 4 mg OA/BS formulation alone. Luminal LPC therefore appears to recruit endogenous lipid into the lymph from non-biliary derived sources (possibly via uptake of lipids from the intestinal blood supply across the enterocyte basolateral membrane) and these basolaterally derived lipids are less able to support lymphatic drug transport when compared with biliary derived lipids (see Fig. 4).

3.5. Mucosal lipid pools and lymphatic drug transport

As described in Section 3.3, there are two mucosal pools of lipid within intestinal absorptive cells. The portal lipid precursor pool consists of a number of discrete lipid droplets distributed throughout the enterocyte cytoplasm and the lipids in this pool are predominantly transported to the systemic circulation via the portal vein. In contrast, the lymph lipid precursor pool consists of lipid droplets destined for transport to the systemic circulation via the intestinal lymphatic system and encompasses lipids within lipoprotein assembly pathways in the ER and Golgi. Due to the close relationship between the sources of lipid and synthetic pathways involved in the formation of the portal and lymph lipid precursor pools (see Section 3.3), the size of the two pools is inter-related. Thus, in the fasted state a relatively small amount of lipid is found in both lipid pools [165], whereas, on administration of exogenous lipids the size and turnover rate of both lipid pools may change significantly. For example, on infusion of 135 μmol/h of triolein (TO) to anaesthetised rats the total mass of lipid contained in both the lymph and portal lipid pools increased 6-fold and the amount of lipid in the portal lipid precursor pool increased 8-fold when compared to the fasted state [140,142]. Interestingly, addition of phosphatidylcholine to the infusion of 135 μmol/h TO reduced the mass of lipid in the portal lipid precursor pool and redirected lipids to the lymph lipid precursor pool. This in turn led to a significant increase in lymphatic lipid output in parallel with the increase in lipid in the lymph lipid precursor pool, suggesting that the size of the portal lipid precursor pool was inversely related to the efficiency of lymphatic lipid output [140,142].

Due to the likely high affinity of lymphatically transported drugs for intracellular lipidic domains we recently initiated a series of studies to examine the potential impact of changes to the size and turnover kinetics of the mucosal lipid pools on the intracellular disposition and lymphatic transport of lipophilic drugs [49]. A steady state lymph-cannulated rat model was employed based on models used previously in the lipid

![Fig. 4](image-url)
biochemistry literature [168]. To the best of our knowledge, however, this was the first time that the technique had been applied to the study of lymphatic drug transport. A series of lipid-based drug formulations (containing radiolabelled fatty acid (FA) and Hf as a model drug) were administered continuously by intraduodenal infusion to lymph-cannulated rats until steady state rates of FA and drug transport into lymph were achieved. Once steady state was achieved the radiolabelled FA and drug (but not other formulation components) were removed from the influsate allowing assessment of the ‘washout profiles’ of FA and drug transport into lymph. The first order rate constants describing FA and drug transport from the lymph lipid precursor pool into the lymph were determined from the washout profiles and the mass of FA and drug in the lymph lipid precursor pool was subsequently calculated from the rate of transport of FA and drug into lymph and the rate constants from the washout profiles. The source of endogenous FA in the lipid pool was also probed by comparing the endogenous lipid output in bile in bile-duct cannulated rats with the rate of endogenous lymphatic lipid flux in animals administered the same formulations.

Following continuous administration of low lipid dose formulations containing a long chain length FA (2 or 5 mg of oleic acid (FA)/h) to steady state, the lymph lipid precursor pool and lymph contained primarily endogenous FA rather than exogenous FA. Consistent with previous results [167], the mass of drug solubilised in the lymph lipid precursor pool following administration of small lipid doses was not related to the total mass of lipid in the lymph lipid precursor pool but rather, appeared to depend on the mass of biliary derived endogenous FA (but not other sources of endogenous FA) in the lymph lipid pool. In contrast, exogenous FA was the major lipid source in the lymph lipid pool and lymph following administration of higher lipid dose formulations (20 mg FA/h) and exogenous FA was the primary driver of lymphatic drug transport. The mass of drug in the lymph lipid precursor pool and available for lymphatic transport was proportional to the mass of total (endogenous plus exogenous) FA in the lipid pool following administration of the higher lipid dose formulations.

Increases in the mass of endogenous and exogenous FA in the lymph lipid precursor pool (e.g. following an increase in lipid dose or on addition of BS and LPC to the formulations), were accompanied by increases in the rate of FA transport into the lymph at steady state. However, the rate of lymphatic FA transport did not increase in direct proportion with the increase in size of the lymph lipid pool. Since the rate of FA transport into the lymph is a product of the mass of FA in the lymph lipid pool and the rate constant describing turnover of FA from the pool into the lymph, the greater fractional increase in the mass of lipid in the pool relative to the rate of transport in the lymph indicated a decrease in the magnitude of the turnover rate constant as the pool expanded. This suggested that FA turnover from the lymph lipid precursor pool into the lymph may have a finite capacity which is saturated as the lymph lipid pool expands. This is consistent with previous studies that have suggested that lipid transport through the enterocyte into the lymph may be limited by the rate at which a transport vesicle which carries premature lipoproteins from the ER to the Golgi buds off from the ER membrane [169].

Increases in the mass of drug in the lymph lipid precursor pool were similarly accompanied by increases in the steady state rate of drug transport into the lymph. However, unlike the data with FA, the first order turnover rate constants describing drug transport from the lymph lipid pool into the lymph were relatively constant, regardless of the mass of lipid administered or the size of the lymph lipid pool. Furthermore, in all cases the rate constants describing drug turnover were lower than the corresponding rate constants for lipid. Given that drug is thought to be transported from the lymph lipid precursor pool into the lymph in conjunction with lipid (i.e. in association with lymph lipoproteins) these data were unexpected and suggested the possibility that drug removal from the lymph lipid pool occurred not only via transfer into the lymph, but also via an additional rate process (hence the lower than expected lymph transport rate constant). Subsequent studies suggested that this additional process was that of enterocyte-based metabolism [170]. The impact of lipoprotein association on enterocyte-based metabolism is described in more detail in Section 3.6.2.

Results from these studies therefore demonstrate that the rate and extent of lymphatic drug transport is dependent on the size and turnover kinetics of the lymph lipid precursor pool and that formulation excipients which expand the lymph lipid precursor pool (such as phospholipids [49,140,142]) may enhance lymphatic drug transport. Whilst this early research suggests that relatively small lipid doses (of a size relevant to the development of lipid-based formulations) may alter the intracellular pooling of lipids and thereby impact on intracellular drug disposition, further research is required to define more carefully how these changes impact on lymph-portal drug partitioning as well as overall absorption and bioavailability of lipophilic drugs.

3.6. Lymphatic drug transport and first-pass metabolism

3.6.1. Hepatic first-pass metabolism

The impact of lymphatic drug transport on hepatic first-pass metabolism has been well described in the literature [11,16]. Briefly, however, lymphatically transported drugs are protected from first-pass hepatic metabolism because the mesenteric lymph, unlike the portal blood, empties directly into the systemic circulation without first passing through the liver. For example, testosterone (T) has extremely limited oral bioavailability due to extensive pre-systemic clearance in the intestine and liver [171,172]. By contrast, testosterone undecanoate (TU) [173–175], a highly lipophilic prodrug of T, is orally bioavailable and exhibits androgenic activity after oral administration. The androgenic activity of orally administered TU is generally attributed to systemic T and the active metabolite 5α-dihydrotestosterone (DHT) which are formed from TU after entry into the systemic circulation via the intestinal lymph [16]. The importance of lymphatic transport of TU to systemic T exposure has been examined in greyhound dogs where following postprandial administration of TU, 83–84% of the systemically available T was found to result from
systemic hydrolysis of lymphatically transported TU [16]. Systemic exposure of T in humans also increases substantially following oral TU administration in the fed state, when compared with administration in the fasted state [176,177]. The increase in postprandial exposure of T, therefore likely reflects both enhanced lymphatic transport of TU and an increase in luminal solubilisation of the poorly water soluble steroid.

3.6.2. Enterocyte-based first-pass metabolism

In addition to hepatic first-pass metabolism, enterocyte-based drug metabolism may also be influenced by drug association with lymph lipoproteins. For example, Vetter et al. examined the impact of enterocyte-based metabolism on the lymphatic transport of benzo(a)pyrene (BP) following oral delivery to killifish [178,179]. After oral administration, BP and lipid were dispersed in the SI lumen, co-transported across the microvillus membrane and accumulated together within the enterocyte. Eventually, however, the lipid was transported from the intestine into the lymph in the form of lipoproteins, whereas BP was dispersed throughout the cell and did not become associated with lipid. The authors suggested that BP was converted to a more hydrophilic metabolite on contact with the metabolic enzymes situated on the SER and therefore that BP was absorbed into the systemic circulation as a metabolite via the portal vein [178,179]. A further study indicated that the formation of larger fat droplets within the enterocyte following a fatty meal reduced transfer of BP from lipid droplets associated with the SER membrane to microsomal enzymes thereby reducing BP metabolism [178].

As described in Section 3.5, data obtained in our laboratories using the steady state lymph-cannulated rat model have also suggested that drug association with lymph lipoproteins in the lymph lipid pool may alter patterns of drug metabolism in the enterocytes. In these studies, the first order rate constants describing Hf transport from the lymph lipid precursor pool into the lymph were significantly lower than the equivalent rate constants describing FA turnover into the lymph suggesting a reduction in the rate of enterocyte-based metabolism [180–182].

### Table 1

|                        | Hf in 5 mg/h OA | DDT in 5 mg/h OA | Hf in 20 mg/h OA/LPC | DDT in 20 mg/h OA/LPC |
|------------------------|----------------|------------------|----------------------|-----------------------|
| Total FA in the lymph lipid precursor pool (μmol) | 23.1±1.5 | 22.8±2.6 | 143.3±13.4 | 139.0±16.2 |
| $K_{D}$ (h$^{-1}$)  | 0.84±0.07 | 0.79±0.03 | 0.46±0.04 | 0.49±0.07 |
| $K_{C}$ (h$^{-1}$)  | 0.43±0.03 | 0.76±0.04 | 0.30±0.04 | 0.47±0.07 |
| $K_{x}$ | 0.48±0.06 | 0.04±0.07 | 0.33±0.08 | 0.04±0.03 |

This table is reproduced with permission [170].

- Statistically different compared to administration of the equivalent formulations containing 5 mg OA (P<0.05).
- Statistically different compared to $K_{x}$ following administration of the same formulation (P<0.05).
- Statistically different compared to $K_{D}$ following administration of the same formulation containing DDT (P<0.05).
- Statistically different compared to $K_{x}$ following administration of the same formulation with Hf (P<0.05).

Turnover into the lymph were not significantly different after administration of either Hf in the presence of KC or DDT, supporting the suggestion that Hf was removed from the lymph lipid precursor pool by enterocyte-based metabolism. These data allowed further examination of the relationship between enterocyte-based metabolism and lymphatic drug transport by using the difference between the first order rate constants obtained for Hf and FA transport into the lymph as an indirect indicator of enterocyte-based metabolism. Re-examination of the data in Table 1 therefore suggests that co-administration of Hf with a larger lipid load (20 mg OA/5.2 mg LPC per h compared to 5 mg OA per h) increases the size of the lymph lipid precursor pool (and increases the extent of Hf lymphatic transport), but also reduces the difference between the first order rate constants for Hf and FA turnover into the lymph suggesting a reduction in the rate of enterocyte-based metabolism of Hf in the presence of a larger lymph lipid precursor pool. The mechanism by which co-administration of increasing quantities of lipid reduced enterocyte-based metabolism was not studied but may reflect the sequestration of drug into larger lipid droplets that are formed in the SER at higher lipid doses in turn reducing drug accessibility to metabolic enzymes located on the SER surface [180–182].

Previous studies have shown that the plasma ratio of Hf metabolite (Hfm) to Hf is lower following administration of Hf with a fatty meal [11] and have further suggested that this is, at least in part, due to avoidance of first-pass hepatic metabolism by stimulating lymphatic transport of Hf. This most recent data [49,170] further suggests that stimulation of lymphatic transport of Hf via co-administration with lipid may enhance bioavailability by avoiding both enterocyte-based and hepatic first-pass metabolism.
4. Summary and future perspectives

The identification of increasingly lipophilic drug candidates has dictated a recent increase in interest in the mechanisms by which drugs access the lymph, the formulation approaches that may be taken to maximise or minimise lymphatic transport, and the potential impact of lymphatic transport on drug processing both within the enterocyte and the liver. Stimulation of intestinal lymphatic transport has potential advantages including a reduction in first-pass metabolism and the delivery of high concentrations of drug to the lymphatic system. Whilst recent studies have increased our understanding of the role of lipid precursor pools in lymphatic drug transport and have started to probe the importance of the source of endogenous lipids that might support lymphatic drug transport, the level of mechanistic understanding of drug access to the lymph at a cellular level remains relatively poor. Further increases in useful application of the intestinal lymph as an alternate mode of transport to the systemic circulation are therefore dependent on studies addressing the fundamental mechanism of drug association with lipoproteins in the enterocyte, and the impact of lipids and formulation excipients on this process.

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