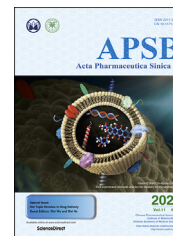




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REVIEW

An update on oral drug delivery *via* intestinal lymphatic transport



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Abstract Orally administered drug entities have to survive the harsh gastrointestinal environment, penetrate the enteric epithelia and circumvent hepatic metabolism before reaching the systemic circulation. Whereas the gastrointestinal stability can be well maintained by taking proper measures, hepatic metabolism presents as a formidable barrier to drugs suffering from first-pass metabolism. The pharmaceutical academia and industries are seeking alternative pathways for drug transport to circumvent problems associated with the portal pathway. Intestinal lymphatic transport is emerging as a promising pathway to this end. In this review, we intend to provide an updated overview on the rationale, strategies, factors and applications involved in intestinal lymphatic transport. There are mainly two pathways for peroral lymphatic transport—the chylomicron and the microfold cell pathways. The underlying mechanisms are being unraveled gradually and nowadays witness increasing research input and applications.

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Abbreviations: ACQ, aggregation-caused quenching; ASRT, apical sodium-dependent bile acid transporter; AUC, area under curve; BCS, biopharmaceutics classification system; CM, chylomicron; DC, dendritic cell; DDT, dichlorodiphenyltrichloroethane; DTX, docetaxel; FA, fatty acid; FAE, follicle-associated epithelia; FRET, Förster resonance energy transfer; GIT, gastrointestinal tract; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; LDV, Leu-Asp-Val; LDVp, LDV peptidomimetic; M cell, microfold cells; MG, monoglyceride; MPA, mycophenolic acid; MPS, mononuclear phagocyte system; OA, oleate; PCL, polycaprolactone; PEG-PLA, polyethylene glycol-poly(lactic acid); PEI, polyethyleneimine; PLGA, poly(lactic-co-glycolic acid); PVA, poly(vinyl alcohol); RGD, Arg-Gly-Asp; RGDp, RGD peptidomimetic; SNEDDS, self-nanoemulsifying drug delivery system; SEDDS, self-emulsifying drug delivery system; SLN, solid lipid nanoparticles; TEM, transmission electron microscopy; TG, triglyceride; TPGS, D- α -tocopherol polyethylene glycol 1000 succinate; TU, testosterone undecanoate; WGA, wheat germ agglutinin; YCW, yeast cell wall.

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1. Introduction

The oral route is usually preferable to other routes for drug delivery owing to safety considerations, convenience of administration, patient compliance, flexibility in dosage adjustment, suitability for long-term use and less strict requirements for oral dosage forms^{1,2}. Orally administered drug formulations should go across the stomach, small intestine and colon in sequence. Drug absorption begins in the stomach but the very short gastric emptying time (0.5–2 h) limits the overall drug absorption extent and only weakly acidic or neutral drugs that present in absorbable molecular form are absorbed in fair amount¹. The small intestine is generally regarded as the major absorption site for a majority of orally administered drug entities. The presence of villi and microvilli lining the intestinal walls enlarges the absorptive surface area to approximately 300–400 m², thus greatly increasing the chances of absorption of numerous molecules^{3,4}. A majority of drug entities entering the gastrointestinal tract (GIT) are absorbed by a passive diffusion or active transport mechanism in common *via* the enterocytes—the main type of cells dwelling on the apical surfaces of the enteric epithelia—and delivered through the portal vein to the liver and finally to the systemic circulation^{5,6}. Commonly, drugs with suitable properties—for example, classification I drugs according to the Biopharmaceutics Classification System (BCS) that possess high solubility and high permeability—are able to achieve high bioavailability and satisfactory therapeutic efficacy^{7,8}.

Despite the above-mentioned advantages, oral drug delivery is confronted with multiple challenges. The harsh gastrointestinal environment owing to secretion of gastric acid, various surfactants and enzymes tends to degrade or inactivate certain labile entities including both small molecular drugs and biomacromolecules^{9–12}. Some therapeutic compounds are stable and absorbable in the GIT, but are susceptible to first-pass hepatic metabolism and suffer from extremely low bioavailability^{13,14}. Moreover, the enteric epithelia may also present as a formidable barrier to the entry of entities of less favorable properties^{15,16}. BCS III (high solubility, poor permeability) and IV (poor solubility, poor permeability) drugs and biomacromolecules fall within this category. Despite advances in formulation and delivery system designs in recent years, oral delivery of labile and poorly permeable therapeutics remains a challenge^{4,17–19}.

In this review, we will address an alternative pathway—the lymphatic pathway—of high promise for oral drug delivery. In contrast with the portal vein-to-liver pathway, lymphatic transport directs drugs first to the lymphatics and finally to the systemic circulation. There are so far two major targets—the chylomicrons (CMs) in enterocytes and the microfold cells (M cells) in Peyer's patches—clearly identified to attain efficient lymphatic transport^{20,21}. Compared with portal transport, lymphatic transport has several advantages: 1) entities absorbed are delivered directly to the systemic circulation *via* the lymphatics and thus circumvent hepatic first-pass metabolism; 2) the leaky capillaries of the lymphatics allow transport of macromolecules and particles that have relatively large sizes; 3) lymphatic delivery holds promise for the treatment of diseases afflicting the lymphatic systems such as human immunodeficiency virus (HIV) infection^{22–24}. Intestinal lymphatic transport has been established as a new alternative platform for oral drug development.

2. Physiology related to enteric lymphatic transport

The intestinal lymphatic system is virtually a unidirectional system that drains interstitial fluids^{25,26}. It is mainly comprised of lymphatics—sophisticated networks of lymph vessels, lymphatic nodes, and lymphoid organs (Fig. 1)²⁷. The enteric lymphatics are generally blind-ended capillaries originating from organs or tissues and then proceed into less branched and more integrated pre-collecting lymphatics, collecting lymphatics, lymphatic trunks and lymphatic ducts in sequence (Fig. 1B)^{27,28}. The collecting lymphatics, termed as afferent and efferent lymphatics for pre-nodal and post-nodal collecting lymphatics respectively, pass through the lymph nodes and converge into the thoracic duct or right lymph duct, and eventually reach the systemic circulation through the subclavian veins (Fig. 1B)²⁹.

The organization of intestinal lymphatic vasculatures is of critical importance concerning drug and particle absorption. It contains mainly two independent subdivisions—one consisting of lacteals and submucosal lymphatics and the other muscular-layered lymphatics³⁰. Each subdivision drains independently and converges concurrently into mesenteric collecting lymphatics^{30,31}. Lacteals and submucosal lymphatics are at the forefront of intestinal lymphatic transport^{32,33}. Lacteals are blind-ended lymphatic capillaries located in the center of each intestinal villus's lamina propria and surrounded by contractible smooth muscle fibers as shown in Fig. 1A,³⁴. Lacteals connect with submucosal lymphatics, which successively converge into mesenteric collecting vessels, lymphatic nodes, cisterna chyli and thoracic duct and finally reach the systemic circulation through jugular and left subclavian vein^{27,30,35,36}. CMs secreted in enterocytes to facilitate lipid absorption are transported through this pathway^{21,37–39}. In comparison with peripheral blood capillaries, which have tight vessel walls, the leaky capillaries of lymphatics with fenestrations commonly of a few tens to hundreds of nanometers allow drainage of macromolecules and particulates of suitable sizes despite the much faster blood flow than lymph flow (500 vs.1)^{40–42} (Fig. 1C).

The M cells-to-lymphatics pathway is another important passage for lymphatic transport. Peyer's patches are a secondary lymphoid tissue mainly distributed in ileum^{24,43} (Fig. 1A). The surfaces of Peyer's patches are covered by follicle-associated epithelia (FAE), and the M cells comprise approximately 10% of the cell population of FAE in murine and less than 5% in human⁴⁴. The function of M cells is to capture particles such as pathogens from the intestinal lumen and transport them to sub-FAE lymphoid tissues, where they are retained and destroyed^{45–47}. Both less coated mucus layers and lower intracellular enzyme activities associated with M cells are beneficial to transport of particles. The particles entrapped in the 'dome trap'—according to Qi et al.⁴⁸—have the opportunities to escape and migrate *via* the lymphatics to the systemic circulation. The lymphatics surrounding the Peyer's patches play an important role in particle transport. Generally, they originate from the lacteals and submucosal lymphatic networks and develop into inter-follicular regions, forming basket-like shape by encircling the medium-basal part of each Peyer's patch^{49,50}. The lymphatics always run along with blood vessels; both of them are abundant in peri-follicular and inter-follicular regions but rare in germinal

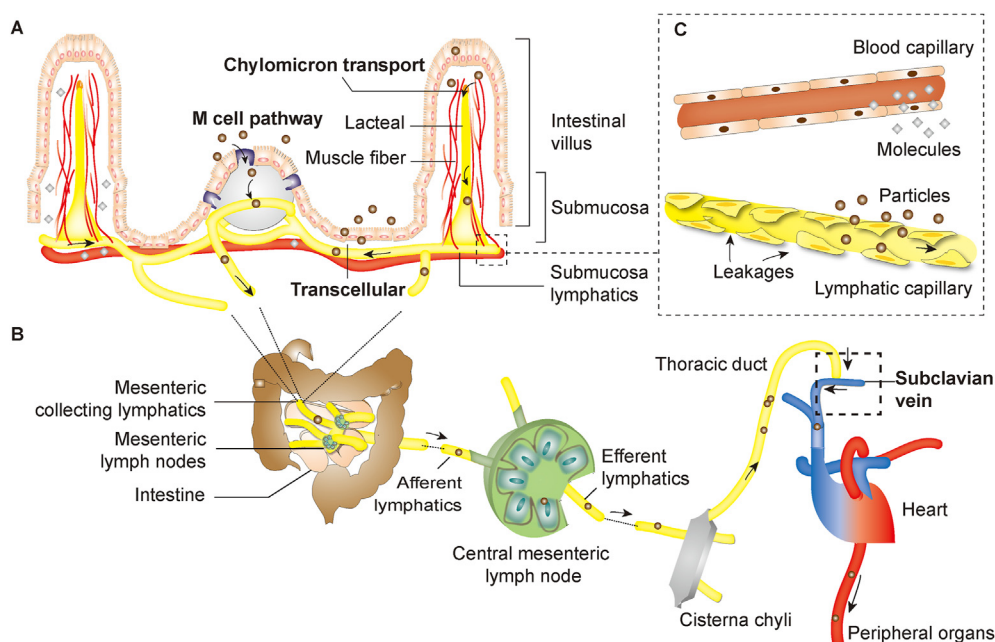


Figure 1 Schematic presentation of the structures of the lymphatic systems involved in transport of particulates and macromolecules. (A) Intestinal lymphatics; (B) Lymphatic circulation; (C) Comparison of blood and lymphatic capillaries.

centers except for a few tiny branches^{51,52}. Each Peyer's patch has a drainage pathway with distinct pre-collectors reaching the same destination as the lacteals⁵². Muscular lymphatics distributing around the superior portion of Peyer's patches may also contribute to drug delivery⁵³. They drain independently and eventually converge into the mesenteric lymph for further transport. Peyer's patches open gates for the entry of particulates, though we still do not know the exact contribution to oral absorption⁵⁴.

Pre-collecting vessels are the initial destinations for lymphatic drainage⁵⁵. Their surface structures are also irregular, discontinuous and similar to small capillaries⁵⁶. Nevertheless, larger lymphatics like lymphatic collecting vessels are not as permeable as these smaller absorptive vessels⁵⁷. They generally have continuous zipper junctions and are covered by complete basement membranes, pumping fluid together with its content—absorbed particles or drug molecules—with valves and smooth muscles lined on the outer side of the vessels^{57,58}. These large lymphatics further converge, to form important immune tissues—lymph nodes. Approximately 100–200 lymph nodes are found in the mesenteric system for proceeding lymphatics into more branchless ones. Central mesenteric lymph nodes are responsible for uniting trunks from different digestive organs to the gastrointestinal trunk⁵⁹. And the gastrointestinal trunk along with lumbar trunk gathers in cisterna chyli, forming the root of thoracic chyle duct⁵⁹. From the thoracic duct, the lymph flows into the systemic circulation *via* the subclavian veins, which commonly involve a single or multiple channels—that is, through the internal jugular vein, through the jugulovenous angle, directly into the subclavian veins or through multiple channels combined⁶⁰.

It should also be noted that the behaviors of particulates in the gastrointestinal lumen may affect later stages of lymphatic drug transport. In the case of lipid-based delivery systems, the vehicles are transformed into secondary lyotropic vesicular and finally micellar vehicles with reinforced mucus-penetrating

ability upon lipolysis by lipases of the constituting lipids in the GIT. The encapsulated drugs should be transferred efficiently without premature release through the sequential structural transformation. In the case of particulates that can be delivered across the mucus and the enteric epithelia intact, the drugs are meant to be secured with the vehicles throughout the whole transportation process.

3. The chylomicron pathway

3.1. Chylomicron structure and transport mechanism

CMs are lipid spheroids formed in enterocytes in response to stimulation of lipid ingestion and digestion to facilitate transport of lipids, especially entities of high lipophilicity such as triglycerides (TG). The common structure of CM is comprised of TG and cholesterol esters in the cores and phospholipids, cholesterol and wrapping proteins on the surfaces (Fig. 2)⁶¹.

Following lipolysis in the intestinal lumen, fatty acids (FA) and monoglycerides (MG), mainly 2-MG, are liberated and subsequently absorbed by enterocytes *via* a mechanism mediated by FA-binding membrane proteins and FA-related transporters dwelling on the apical membrane surfaces^{20,62–64}. Long-chain FAs are able to be re-esterified and bind with lipoprotein—mainly apolipoprotein B—to form pre-CM⁶⁵. After a series of endocellular processes, pre-CMs mature into CMs which are secreted into the mesenteric lymph and thereafter transferred *via* the lymphatics into the systemic circulation⁶⁶. In the blood, with the aid of lipoprotein lipases, TGs constituting the CM cores are partially hydrolyzed while CMs evolve to form CM remnants which finally turn to the liver, bind with low-density lipoprotein (LDL) receptors or LDL receptor-related proteins, and are removed by lipoprotein lipases and hepatic lipase E^{66,67}.

3.1.1. Prerequisites for drug transport via CM

It is envisioned that entities having CM-binding capacity may take advantage of the CM pathway to be delivered *via* the lymphatic route. Empirically, only a few highly lipophilic molecules with $\lg P$ values more than 5 and long-chain TG solubility more than 50 mg/g are transported *via* the CM pathway^{20,68}. When lipid-based vehicles are involved, the vehicles are usually degraded first through lipolysis, transformed into micellar vehicles, absorbed by enterocytes and re-assembled with CM⁶⁹.

$\lg P$ and TG solubility are the most frequently reported key factors for predicting drug lymphatic transport. Numerous drugs have been screened to testify these critical parameters. The lymphatic absorption of dichlorodiphenyltrichloroethane (DDT) with a $\lg P$ of 6.19 and TG solubility of 80 mg/g and halofantrine with a $\lg P$ of 8.5 and TG solubility >50 mg/mL was found to be 15% and 20% of the initial dose, respectively⁷⁰. Vitamin D₃ and E, which meet the $\lg P$ and TG solubility standards, exhibited high CM binding percentage (>50% of the original dose) and lymphatic delivery efficiency (15%–20% of the original dose)⁷¹. However, it is inaccurate to conclude that $\lg P$ and TG solubility are the sole parameters for prediction of lymphatic transport⁷². Some studies give contradictory results. For example, both penclofedine ($\lg P$ 5.48, TG solubility 175 mg/g) and CI-976 ($\lg P$ 5.83, TG solubility >100 mg/g) match the $\lg P$ and TG solubility criteria but indicate low lymphatic exposure⁷⁰. A retrospective study revealed poor correlation between lymphatic bioavailability and $\lg P$ or lipid solubility but high correlation with drug–CM affinity⁷¹. In addition to $\lg P$ and TG solubility, factors such as hydrogen binding acceptors, polar surface area and pK_a , among others, all play an indispensable role in CM binding and lymphatic drug transport^{73,74}.

3.1.2. Critical influencing factors

Lipid type (*e.g.*, chain lengths and saturation degree) is reported to be one of the essential factors that influence CM transport. The lipophilicity of short-, medium- and long-chain fatty acids is positively correlated to chain lengths, so is the CM binding capacity. It is reported that short-chain fatty acid prefers transport

via the portal vein, whereas medium- or long-chain fatty acids are prone to be transported *via* the lymph⁷⁵. Recovery of more self-nanoemulsifying drug delivery systems (SNEDDS) consisting of long-chain than medium-chain fatty acids in lymph adds evidence to support the chain length dependency⁷⁶. An investigation on lipid-based vehicles with extraordinarily lipophilic halofantrine as a model drug indicated that the lymphatic affinity of the vehicles followed the order of C₁₈ (15.8%)>C_{8–10} (5.5%)>C₄ (2.22%)>C₀ (0.34%)⁷⁷. Increase in FA chain length correlates well with the higher drug transport efficiency. This could be attributed to the higher lipophilicity of long-chain FAs which have higher affinity to intracellular CMs and lipoproteins. Administered in an oil solution of 1,3-dioctanoyl-2-linoleyl-*sn*-glycerol, encoded as MLM to indicate the position of “medium–long–medium” chains, leads to increased portal absorption of halofantrine but similar levels of lymphatic transport as compared with the sunflower oil solution⁷⁸. Nevertheless, following formulation of halofantrine into SNEDDS based on MLM and 1,3-dilinoyl-2-octanoyl-*sn*-glycerol, encoded as LML to indicate “long–medium–long” chain lengths, the lymphatic transport was found to be 17.9% and 27.4%, whereas the plasma availability was 56.9% (MLM) and 37.2% (LML), respectively⁷⁹. It is implied that proper design of chain lengths and proportions may be a possible measure to alter the drug distribution between different pathways.

The food effect is another factor that may influence lymphatic transport and thereby plasma bioavailability. The oral bioavailability of a radio-labeled cannabinoid receptor agonist CRA13 is approximately 72%–75% in fed dogs vs. 8%–20% in fasted ones, with 43.7% through lymphatic transport⁸⁰. Administration after a meal increases the total amount of lymphatic transport of halofantrine from 1.3% (administered before a meal) to 54% of the administered dose⁸¹. Sometimes, food intake may delay the absorption process but not necessarily alter the AUC and systemic bioavailability⁸². It should be noted that there are exceptions with regard to the food effect. For example, after ingestion of a high-fat meal, the AUC of DDT with high CM binding efficiency increases by 1.5-fold, whereas no significant difference is observed for diazepam with low CM binding efficiency under the same

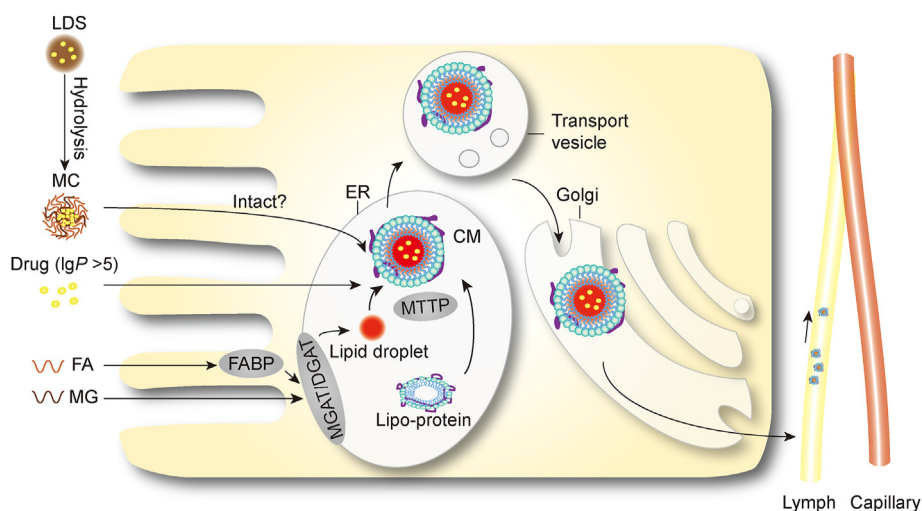


Figure 2 Schematic presentation of the formation of chylomicrons (MC: micelle; LDS: lipid-based delivery system; FA: fatty acid; MG: monoglyceride; FABP: fatty acid binding protein; MGAT/DGAT: monoacylglycerol acyltransferase/diacylglycerol acyltransferase; MTTP: microsomal triglyceride transfer protein; ER: endoplasmic reticulum; CM: chylomicron). It is still unclear whether micelles could be taken up into the enterocytes intact.

conditions; the lymphatic transport is higher for DDT but not for diazepam⁸³. The food effect may be partly attributed to stimulation of CMs⁸⁴. Ingestion of not only fat but also high contents of carbohydrates contribute to higher CM production⁸⁴. Moreover, lipid ingestion induces secretion of bile salts which take part in formation of mixed micelles together with MGs and drugs, thus facilitating drug uptake and CM assembling within the enterocytes^{85,86}. The observation of significantly reduced halofantrine output in lymph in bile duct-cannulated rats highlights the important role of multiple endogenous bile salts^{87,88}.

3.2. Approaches for enhancement of chylomicron transport

3.2.1. Prodrug approaches

Designing of lipophilic prodrugs is an efficient approach to improve drug solubility in fat and thereby oral bioavailability owing to enhanced lymphatic transport^{89–91}. The basic principle is to impart drug molecules with lipophilicity by conjugating to FAs, MGs or phospholipids^{21,92}. The designed prodrugs enter the enterocytes, associate with lipoproteins, transport *via* the lymphatics and finally reach the systemic circulation in CM-encapsulated form⁹³. In the case of testosterone, a hormonal drug with significant first-pass metabolism, its glyceride-mimicking prodrug bridged with a glyceride through self-immolative spacers remarkably enhances the plasma exposure of testosterone up to 90-fold that of testosterone undecanoate (TU), a commercial testosterone product³⁹. By collecting the mesenteric fluid from lymph-cannulated rats, prodrugs could be recovered in as high as 28% of the administered dose in comparison with 0% for pure testosterone and 1.9% for TU³⁹. This verifies the high lymphatic transport efficiency of the triglyceride-mimetic prodrug strategy. The significantly enhanced plasma concentration and AUC of free testosterone are indicative of the contribution of lymphatic transport.

3.2.2. Oil induction to stimulate chylomicron production

Oil ingestion has been known to induce the formation and secretion of CMs, which may be employed to facilitate lymphatic transport of co-administered or pre-administered drugs, provided that the drugs readily associate with CMs upon contact^{83,94}. An immunomodulator tacrolimus, for example, could achieve 15 times higher absorption rate constant in lymphatics *vs.* blood following delivery with cacao or sunflower butter together⁹⁵. Moxidectin, if co-administered with sunflower oil, could realize 98% increase in bioavailability as a result of enhanced intestinal lymphatic transport⁹⁶. Moreover, both lymphatic output and transport efficiency of lutein could be significantly enhanced when administered with safflower oil and certain monoglycerides or diglycerides as vehicles⁹⁷. The oil type may influence the CM production and lymphatic drug transport process. Among the above mentioned oil types, safflower oil performed the worst for lymphatic transport of lutein, while the others separately achieved 70%–211% improvement in comparison with the safflower group⁹⁴. Induction of CM by oil ingestion could be reinforced by increasing the oil dosage, but not necessarily in a linear correlation and with possible saturation⁹⁸. Fat saturation affects the number and size of CMs produced^{84,94}. The numbers may eventually influence the output of cargoes, while the size may affect CM metabolism in the systemic circulation^{99,100}. For soybean oil, its dose effect on carotenoid absorption is only predominant from 0 to 32 g; beyond that, no significant enhancement effect can be observed. For more lipophilic drug halofantrine, the absorption

may be limited when the oil dose exceeds 250 mg/kg and reaches a plateau when the oil dose reaches 1000 mg/kg¹⁰¹.

It is of note that not all types of oils benefit lymphatic transport of the payloads. For instance, sesame oil limits the absorption of cholesterol by inhibiting the micellar solubilization process^{102,103}. Corn oil and poly-unsaturated fatty acid such as linoleic acid or linolenic acid disfavor absorption of some carotenoids probably due to the poor lipolysis capability and lower extent of micellization^{104,105}. Therefore, it is important to select a proper type of oil for higher lymphatic transport efficiency.

3.2.3. Lipid-based delivery system

Though oil ingestion promotes lymphatic absorption of drugs to some extent, *in vivo* dispersity may still pose a hurdle for more efficient drug absorption¹⁰⁶. Hence, lipid-based delivery systems are designed and several lipid-based carriers such as nano/micro emulsions, self-emulsifying drug delivery system (SEDDS), solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and liposomes all have shown increased bioavailability for various drug compounds by promoting lymphatic transport^{15,107,108}. Upon lipid hydrolysis, FA, MG and bile salts form drug-loaded mixed micelles together. The formed micellar structures can be taken up intact by enterocytes or disassociate to release the payloads. Then free molecules are able to be transported *via* the portal vein, while the particles may associate with CMs and then be transported *via* the lymphatics^{109,110}. These vehicles are trafficked to endoplasmic reticulum and Golgi complexes *via* the apical recycling endosome or common recycling endosome routes after internalization, subsequently stimulating the formation of CM and exocytosis of the carriers^{111,112}.

Despite numerous results in support of lipid-based carriers for enhancement of lymphatic transport and systemic bioavailability, controversies exist with regard to transport mechanism because of the complexity of gastrointestinal physiology and presence of multiple transport pathways^{113,114}. CM flow suppressors such as cycloheximide which inhibit the synthesis of proteins and specifically block the transport of CM have been utilized to evaluate the contribution of CM transport¹¹⁵. For example, the peak plasma concentration (C_{max}) and AUC of raloxifene hydrochloric acid, as encapsulated in SLNs, decrease by 34% and 29%, respectively after pre-treatment with cycloheximide¹¹⁶. For docetaxel lipid-based nanocapsules, nearly no drugs could be detected in plasma with cycloheximide pre-treatment¹¹⁷. Significant decrease in C_{max} and AUC was also observed for carvedilol liposomes as a result of cycloheximide pretreatment, confirming the contribution of the CM pathway to overall bioavailability¹¹⁸.

Additionally, other methods have been adopted for assessment of the CM pathway. Electron microscopy was utilized to visualize the formation of CM in a Caco-2 cell model¹¹⁹. Formation of CMs of sizes ranging from 70 to 150 nm on the basolateral side was observed following oral administration of lipid-based polymethoxyflavones particles with three-fold enhancement of absorption¹¹⁹. Lymphatic ligation is an alternative method, but it should be noted that lymph collected this way may be a mix that includes the lymph derived from the M cell pathway¹²⁰. The monitoring of TGs and drugs in lymph provides indirect information to help explain the transport mechanisms. The fact that saquinavir delivered with Cremophor/oleic acid mixed micelles, D- α -tocopherol polyethylene glycol 1000 succinate (TPGS)/oleic acid mixed micelles and oleic acid microemulsions has the same transporting rate as TG may work to support that the drug was delivered in association with

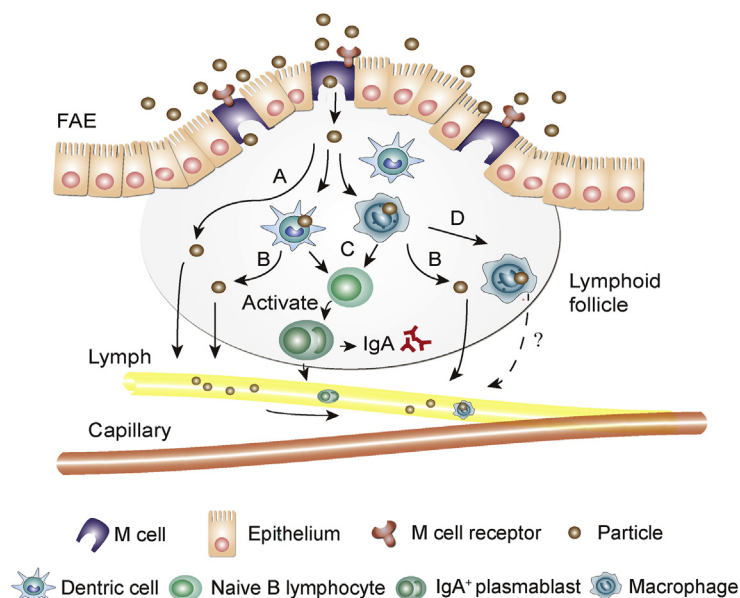


Figure 3 Schematic presentation of cross-section structure of the Peyer's patch and the M cell transport pathway. (A) Particles bypassing immune cells; (B) Particles escaping from immune cells; (C) Particles entrapped in dendritic cells or macrophages inducing immune response; (D) Particles being entrapped within macrophages.

CMs¹²¹. However, the fraction of lymphatic transport only accounts to 0.025%–0.05% bespeaks the limited or negligible contribution of lymphatic transport. The finding was echoed by Guan et al.'s results¹²² that the cumulative percentage of lymphatic transport was only 0.25% and 0.73% for cyclosporine A delivered by liposomes and bilosomes, respectively. It is implied that though lipid-based vehicles facilitate lymphatic drug transport, the contribution of lymphatic transport to overall bioavailability may be negligible for some drugs. Overall, there are controversies regarding the underlying mechanisms of facilitated lymphatic transport by lipid-based vehicles. It seems that multiple mechanisms may be involved.

4. M cell pathway

4.1. Transport mechanism

Peyer's patches are important lymphoid tissues in the gut. The epithelia on the follicle surfaces, termed as FAE, takes a "dome shape"^{23,123} (Fig. 3). The M cells located in FAE are capable of taking up and transporting antigens from the lumen side to the underlying lymphoid follicles where they are eliminated through induction of immune responses^{43,124}. The FAE are also responsible for uptake of a small fraction of orally ingested particulates; this is always regarded as a potential portal for the entry of particulates into the systemic circulation¹²⁵. Although M cells merely occupy approximately 10% of the cell population of FAE in murine and FAE is mainly located to the ileum, the high transcytosis capacity of M cells compensates for the limited cell population^{126,127}.

Following uptake by M cells, antigens or pathogens are transported to the pockets located to the other side of the M cells, where antigen-presenting dendritic cells dwell and keep alert to take over^{128,129}. After antigen sampling and presenting, immune responses are initiated. With regard to the fate of non-antigenic

particulates, there are several possibilities: 1) bypassing immunocytes; 2) escaping immunocytes while eliciting immune responses; 3) escaping immunocytes without eliciting immune responses; 4) encampment and ending up in immunocytes²³ (Fig. 3). It is not hard to make a judgment on the fate of internalized particulates—ending up in the immune system while eliciting immune responses simultaneously, or being transported to distant organs and tissues. Researches have been done to track the translocation of particles. For instance, bioengineered glucan microparticles, or yeast cell wall (YCW) microparticles, are able to remain intact for extended times after uptake by macrophages and distribute in organs of the mononuclear phagocyte systems (MPS) such as the liver, lungs and spleen¹³⁰. Another study¹³¹ reported that microparticles could migrate to MPS organs or macrophage-enriched inflammatory tissues *via* lymph with the aid of macrophages as delivery vehicles. However, factors that influence transport *via* the M cell pathway are still inexplicit, except for the observation that larger particle size favors accumulation, rather than escape, of particulates in Peyer's patches¹³². The M cell pathway presents as a potential portal for particulates to enter the systemic circulation and be subsequently delivered to distant organs or tissues with targeting promises. Importantly, lymphatic transport is also of high interest for oral vaccination or treatment of diseases inflicting the lymphatic systems¹³³.

4.2. Strategies for the M cell pathway

As discussed above, the M cells readily recognize pathogens through recognizing the specific ligands presenting on the surfaces of pathogen particles. Therefore, particles can be engineered to mimic specific ligands to reinforce uptake and transport through this pathway. M cells also take up unadorned particles but probably in less amount and a non-specific way. Active targeting to M cells based on decoration with ligands, such as peptide/protein, non-peptide/protein and microbe-derived

ligands, proves to be an efficient strategy for enhancement of lymphatic transport of particulates^{134–137}. The mostly investigated ligands include lectins, peptidic ligands such as RGD (Arg-Gly-Asp) and glucans.

Lectins, mainly extracted from plants, bind specifically to the carbohydrate residues of proteins or lipids on M cell surfaces¹³⁸. *Ulex europaeus* I (UEA1), for instance, specifically recognizes α -linked fucose residues and binds nearly exclusively to the apical surfaces of M cells¹³⁹. Wheat germ agglutinin lectin (WGA), another member of the lectin family that has better stability and lower immunogenicity, facilitates nanoparticle uptake and transport with more efficiency and safety^{140,141}. Decoration of liposomes with WGA stabilizes the vehicles, which brings about extra benefits for labile payloads¹⁴². However, it is of note that WGA does not target M cells only—it also binds and facilitates penetration of particulates across the biomembranes *via* enterocytes¹⁴³. Tomato lectins bind M cells too, but it is not a good candidate for M cell targeting owing to its overly high affinity with villi¹⁴⁴.

Peptidic ligand RGD recognizes $\alpha 5 \beta 1$ integrin overexpressed on M cells^{136,145,146} but undergoes gradual degradation in the GIT, thus calling for synthesis of more stable non-peptidic analogues such as RGD peptidomimetic (RGDp)¹⁴⁷. RGDp-decorated poly (lactic-co-glycolic acid) (PLGA) nanoparticles was prepared to testify the efficacy of non-peptidic ligands¹⁴⁸. It is observed that RGDp has the same affinity with M cells as RGD but reinforced therapeutic efficacy owing to improved stability in the GIT¹⁴⁷. The pair of LDV (Arg-Gly-Asp) and LDV peptidomimetic (LDVp) is another similar example^{22,149}.

YCW is primarily composed of β -1,3-D-glucan that binds with dectin-1 expressed on M cells and its submucosal macrophages¹⁵⁰. Using YCW microparticles to devise nano-in-micro carriers achieves dual targeting to M cells on FAE surfaces and macrophages in sub-FAE pockets, leading to high drug accumulation in Peyer's patches¹⁵¹. Interestingly, bioimaging to reveal the M cell transport pathway could be achieved by electronically loading quantum dots or organic fluorescent nanoparticles into YCW microparticles¹⁵¹. Flagellin from *Salmonella enteritidis* was also selected as superior ligands to decorate nanoparticles to achieve higher immune responses¹⁵².

Other M cell specific markers such as glycoprotein 2, claudin 4, C5aR (C5a receptor), PrP^C (cellular prion protein) and annexin A5 have shown potential to be applied in M cell targeting as well^{153–156}.

4.3. Factors influencing M cell uptake

Except for active targeting strategy, some physicochemical properties including particle size, surface hydrophobicity, surface charge and particle shape significantly influence M cell uptake¹⁵⁷. These factors must be taken into account when designing delivery systems.

4.3.1. Particle size

Particle size is one of the critical characteristics that affect the *in vivo* fate of nanoparticles. The size effect was investigated by tracking the translocation of polycaprolactone (PCL) nanoparticles labeled by near-infrared fluorescent probes with aggregation-caused quenching (ACQ) properties^{48,158}. After oral administration, more particles of the 600 and 2000 nm groups were transported *via* the lymphatics than the smaller size groups (50 and 200 nm), evidenced by quantification of particles in lymph

collected through mesenteric lymphatic duct cannulation^{48,158}. The particle size effect of nanocrystals was investigated by employing similar ACQ-based bioimaging strategy. Following oral administration of nanocrystals hybridized with ACQ probes, nanocrystals with larger sizes (550 and 1100 nm) showed higher retention in Peyer's patches probably due to the slower dissolution rate and more integral structures as compared with the smaller ones (280 nm)¹⁵⁹. Similar results were observed for cyclosporine A ultrafine particles with 550 nm particles being taken up by M cells in more amount than 250 nm particles¹⁶⁰. More findings concerning glucan, latex and organosilica particles add more evidence to support size dependency in lymphatic transport^{161–163}. It seems that within a certain limit—for example, less than 3 μ m, larger particles tend to be stuck in Peyer's patches due to various reasons¹⁶⁴. After uptake by M cells, particles below 1 μ m can be transported to lymphatics and systemic circulation, while particles larger than 5 μ m are mainly trapped in Peyer's patches^{165,166}.

Notwithstanding numerous researches on this topic, there are still controversies with regard to the effect of particle size and distribution^{167,168}. A study on organosilica particles concluded that 95–200 nm nanoparticles were more optimal for Peyer's patches¹⁶³. It is hard to compare the findings reported in different articles because of utilization of different animal models, material diversity and different methods employed¹⁶⁹.

4.3.2. Surface hydrophobicity

PEGylation is an efficient measure to promote mucus-penetrating ability of nanoparticles and thus enhance oral absorption¹⁷⁰. However, M cells with thinner mucus coating are more preferable for particles with hydrophobic surfaces³. Coating rifampicin-gantrezAN-119 nanoparticles with a hydrophobic polymer ethyl cellulose leads to enhanced absorption of the particles, as evidenced by histological images¹⁷¹. Similar results of enhanced bioavailability and distant organ accumulation were observed with rifampicin gantrez nanoparticles coated with another hydrophobic polymer—polyethylene sebacate¹⁷². Another explanation for higher M cell transport efficiency is that the relatively hydrophobic surfaces favor protein binding in the intestinal fluid, which in turn affects its transcytosis process¹⁷³.

4.3.3. Surface charge

It is reported that positively charged particles are easier to be taken up than negatively charged ones owing to the electrostatic affinity with intestinal mucus or cell membranes¹⁷⁴. The systemic and lymphatic exposure of positively charged polyethylene glycol-poly (lactic acid) (PEG-PLA) nanoparticles is higher than neutral and negatively charged particles, though the lymphatic pathway is not predominant¹⁷⁵. Decoration of SLNs with positively charged hydroxypropyl trimethylammonium chloride chitosan enhances M cell uptake and accumulation in Peyer's patches¹⁷⁶. The same conclusion was drawn for positively charged chitosan-coated liposomes as vehicles for DNA vaccine delivery as well^{177,178}. Notably, there are also controversial reports on the effect of surface charges. For instance, it was reported that neutral surfaces performed better for accumulation of 130 nm and 950 nm polystyrene particles in Peyer's patches³, while negatively charged poly (vinyl alcohol) (PVA)-coated PLGA nanoparticles showed higher accumulation in Peyer's patches compared to polyethyleneimine (PEI)-coated positively charged ones¹⁶⁴. Negatively charged gold nanoparticles had higher absorption than positively charged ones¹²⁷. All these results imply that there may be

interplay between surface charges and other factors such as hydrophobicity, stability, and lipolysis rate¹⁷⁹.

4.3.4. Particle shape

Particle shape is an important physicochemical factor that influences multiple biological behaviors of drug delivery system *in vivo*. Regarding oral delivery, variation in particle shape leads to deviated biological interactions, which result in deviated dissolution, permeation, cellular translocation, distribution and ultimately drug bioavailability and therapeutic effect¹⁸⁰. For instance, the M cell uptake efficiency is subjected to particle shape variations, as evidenced by cellular uptake and trans-monolayer transport in Caco-2/Raji-B cell models¹⁵⁷. Results showed that rod- and disc-shape particles achieved 15% and 18% transport of total particles, respectively, while the spherical ones only reached a maximum of approximately 11%. The shape effect was also proved by *in vivo* investigation¹⁸¹. Compared to the spherical-shaped particles (0.98%), rod-shaped particles attained higher extent of lymphatic transport (1.75%), as evidenced by semi-quantification based on Förster resonance energy transfer (FRET) bioimaging. Nevertheless, the underlying mechanisms behind the shape effect remain elusive and it is difficult to evaluate this sole parameter without altering other factors¹⁸².

5. Paracellular and transcellular pathways

In addition, both the paracellular and transcellular pathways whose role in lymphatic drug transport has not been clearly identified also hold promise for further investigation. Intercellular tight junctions that are built of multiple protein complexes constitute the main barriers to drug transport *via* the paracellular route¹⁸³. Many absorption enhancers like ethylenediaminetetraacetic acid (EDTA), chitosan, bile salts, sodium caprate and some synthetic peptides are able to open tight junctions, thus creating tentative apertures for the entry of macromolecules and particles of appropriate sizes^{184,185}. By co-delivery of insulin with an absorption enhancer sodium caprate in microcontainers, the oral bioavailability of insulin could be enhanced partially owing to the contribution of the paracellular route¹⁸⁶. Generally, the paracellular apertures only allow the transport of macromolecules with suitable particle sizes, such as insulin with a hydrodynamic radius of approximately 2 nm at pH 7.4 and fluorescently labeled dextran with a Stokes radius of approximately 1.3–2 nm^{187,188}. However, it is controversial whether particles with larger sizes are able to penetrate the paracellular passages. A report suggested that 5 nm gold nanoparticles could transport through cellular intervals¹⁸⁹. Even polyisobutyl cyanoacrylate nanocapsules with a size of approximately 150 nm could be observed in intercellular spaces of the intestinal villi tip by SEM^{190,191}. However, evidence showed that the maximum pore size could only be expanded to approximately 20 nm even with efficient permeation enhancers¹⁹². It is highly probable that intact particles could hardly pass across the intercellular barriers.

The fate of macromolecules or particles beyond enterocyte uptake may be different. It is reported that macromolecules such as insulin and GLP-1 are preferably transported through the portal vein, thus being able to imitate the secretion under physiological conditions¹⁹³, while particles with larger sizes are prone to be transported further *via* the lymphatic capillaries owing to the obstruction by blood vessel walls¹⁹⁴. Fluorescently labeled

dextrans of a series of molecular weight provide a useful tool to test this hypothesis. The transport *via* lymphatics overwhelms that *via* blood vessels when the molecular weight of dextrans increases to above a threshold¹⁹⁵. However, it should be attributed to reduced uptake by blood capillaries, rather than elevated transport *via* lymphatics¹⁹⁵.

Another important absorption pathway is the transcellular pathway. Though it is a general route for oral absorption of small molecules, the possibility of absorption of particles through this pathway should not be excluded. If nanoparticles are able to be absorbed intact, there are chances for them to be transported into the systemic circulation, and they may preferably choose the lymphatic pathway because of the fenestrated walls of the lymphatics¹⁹⁶. This is especially the case for small-sized PCL nanoparticles (50 nm) which are taken up more profoundly by *in vitro* Caco-2 cell models and transported more efficiently *via* the lymphatics¹⁵⁸. Except for this leakage-aided process, receptor-mediated transport *via* lymphatics was also proposed¹⁹⁷. High lymphatic absorption of exenatide achieved by dextran-coated particles may have taken advantage of the specific binding between dextran and receptors expressed on the surfaces of lymphatics^{198,199}.

Notably, all the transport pathways mentioned in this part are difficult to verify because of a lack of dynamic monitoring and reliable quantification measures. Nevertheless, all of them may play a role in oral absorption of biomacromolecules and particulates because of the large absorption area and preliminary observation of intracellular behaviors.

6. Application of lymphatic transport in drug delivery

Based on increasing understanding of involved mechanisms, lymphatic transport has become an important approach in drug discovery. Table 1^{39,171,200–229} summarizes examples of application of lymphatic transport in oral drug delivery for reduced first-pass effect, facilitated oral absorption, distant organ targeting and lymphatic associated treatment.

6.1. Reduction of first-pass effect

As the mesenteric lymph flow circumvents the portal vein, drugs transported *via* the lymphatic route do not subject to first-pass hepatic metabolism. Therefore, structural modification to render them amenable for lymphatic transport has become an attractive option for entities with severe first-pass metabolism—for example, testosterone and docetaxel. The oral bioavailability of testosterone is too low to elicit any therapeutic effect. Hence, substituted, long-chain ester TU is marketed for clinical use as an oral dosage form due to significantly enhanced lymphatic transport and reduced liver metabolism. The absolute oral bioavailability of two commercial TU products was increased to approximately 3.25% and 2.88%, for which the contribution of lymphatic transport was approximately 91.5% and 99.7%, respectively, as revealed in a study in thoracic lymph duct-cannulated dogs²⁰⁰. However, the long-chain ester prodrug strategy is not without concerns—the balance between stability of the ester derivatives *in vitro* and cleavage rate *in vivo* sometimes poses a challenge. To address this, testosterone-triglyceride prodrugs were designed to improve the stability of testosterone and elevate its systemic exposure in rabbits with AUCs enhanced by 1.8 and

Table 1 Summary of application of intestinal lymphatic transport.

Model drug	Prodrug/vehicle	Result	Ref.
Circumvent first-pass metabolism			
Testosterone	Testosterone undecanoate	Absolute oral bioavailability (% lymphatic transport) of two TU products: 3.25% (91.5%); 2.88% (99.7%)	200
Testosterone	TST-TG	AUC: 1.8- and 2.6-fold higher than TU at 8 and 4 mg/kg oral dose	201
Testosterone	TST-acetal self-immolative group-TG; TST-trimethyl group-TG	AUC: 10–90-fold higher than TU	39
Docetaxel	DTX-S-OA	Bioavailability: 6.2- and 2.0-fold higher than DTX solution and DTX SNEDDS	202
Docetaxel	DTX-S-S-TG	Absolute bioavailability: 44.3%; relative bioavailability: 470.7% vs. DTX solution)	203
Quercetin	Emulsions	Higher lymphatic delivery and higher systemic exposure of both quercetin and its metabolites	204
Nitrendipine	Solid lipid nanoparticles	Bioavailability: 3.09–3.93 times higher than its suspension	205
Berberine chloride	Creomchylomicron (Cremophor EL, Tween 80)	43% reduction in absorption after pre-treatment with cycloheximide	206
Carvedilol	Microemulsions	2.84–4.91-fold enhanced in bioavailability	207
Olanzapine	Nanostructured lipid carriers	5.5-fold increase in bioavailability vs. suspension	171
Lopinavir	TG-mimetic 1,3-dipalmitoyl glycerol-decorated mesoporous silica nanocarriers	C_{max} : 1.69-fold increase; AUC: 5.97-fold increase (vs. free lopinavir)	208
Facilitated oral absorption by particulates			
Probucol	SNEDDS	10.2-fold improvement in bioavailability	209
Baicalin	Nanoemulsions	AUC: 14.56-fold increase; 26.8% decrease after pre-treatment with cycloheximide	210
Solvent Green 3	SNEDDS	Absorption was totally inhibited after pre-treatment with cycloheximide	211
curcumin	<i>N</i> -Carboxymethyl chitosan coated solid lipid nanoparticles	Higher lymphatic transport and bioavailability	212
Silybin	Silybin-phospholipid; SB-PC SNEDDS	Relative bioavailability: 1265.9% (SB-PC); 1802.5% (SB-PC SNEDDS). Lymphatic exposure: 12.2 (SB-PC) and 22.7 (SB-PC SNEDDS)-fold higher than pure SB	213
Candesartan cilexetil	Solid lipid nanoparticle	Bioavailability: 12-fold increase vs. CC suspension; AUC: 30% decrease after cycloheximide treatment	214
Morin	Phospholipid complex-based SNEDDS	Multiple absorption pathways were observed including the M cell and CM pathways	215
Lutein	Solid dispersions and SMEDDS	Enhanced lymphatic transport efficiency was achieved; different absorption rates between two formulations were observed	216
Insulin	Polymeric nanoparticles	Relative bioavailability: 13.21%; high Peyer's patch accumulation	217
Insulin	Glucan microparticles	Pharmacological bioavailability: 9%–10%; good correlation between lymphatic transport and pharmacological bioavailability	218
Insulin	Chondroitin sulfate-taurocholic acid conjugate-decorated liposomes	Chylomicron transport was observed	219
Exenatide	Phase-changeable nanoemulsions	Higher drug accumulations were observed in pancreas; drug absorption and lymphatic transport were inhibited by cycloheximide	220
Distant site targeting			
Paclitaxel	WGA-decorated SLNs	Lung targeting	221
Cisplatin	YCW microcapsules	Lung tumor targeting	222
Sorafenib	Sugar-grafted SLNs	Liver tumor targeting	223
Vinpocetine	Mixed micelles	Brain targeting	224
Treatment involving the lymphatic system			
Ovalbumin	Mannose- and flagellin-decorated Polyanhydride nanoparticles	Enhanced and balanced Th1/Th2 response; high intestinal IgA level	225

(continued on next page)

Table 1 (continued)

Model drug	Prodrug/vehicle	Result	Ref.
HBsAg	Lectin-decorated PLGA nanoparticles	Higher anti-HBsAg antibody levels and enhancing mucosal immunity	226
HBsAg	UAE 1-decorated liposomes	Comparable IgG level with intra-muscular HBsAg following immunization for three consecutive days	227
Mycophenolic acid	Triglyceride mimetic prodrug	103-fold higher concentration in lymphocytes for prodrugs and 28-fold higher in lymph nodes for mycophenolic acid	228
Bexarotene and retinoic acid	Ester prodrug	17- and 2.4-fold higher concentration in lymph nodes vs. free drug	229

TU, testosterone undecanoate; TST, testosterone; TG, triglyceride; DTX, docetaxel; DTX-S-OA, docetaxel-sulfur-oleate; DTX-S-S-TG, docetaxel-sulfur-sulfur-triglyceride; SB, silybin; SB-PC, silybin-phospholipid; CC, candesartan cilexetil; EXT, exenatide; WGA, wheat germ agglutinin lectin; SNEDDS, self-nanoemulsifying drug delivery systems; SMEDDS, self-microemulsifying drug delivery system; SLNs, solid lipid nanoparticles; CM, chylomicron; YCM, yeast cell wall microparticles; PLGA, poly(lactic-co-glycolic acid); HBsAg, hepatitis B surface antigen.

2.6 times as compared with TU at a dose of 8 and 4 mg/kg, respectively²⁰¹. Further insertion of self-immolative spacers—acetal self-immolative group and trimethyl group for “locking”—into the prodrug structure ensures fast cleavage of the ester bonds and instant release of testosterone in the blood stream with 10–90-fold increase in AUC after oral administration³⁹. Conjugation of docetaxel (DTX), an anti-cancer drug with pronounced hepatic metabolism, with oleate through a thioether bond (DTX-S-OA) increases the lipophilicity of DTX and thereby drug loading in SNEDDS²⁰². The oral bioavailability of DTX-S-OA is 6.2- and 2.0-fold higher than DTX solution and SNEDDS, respectively²⁰². Additionally, by conjugating DTX with triglyceride through a reduction-sensitive disulfide bond, the absolute bioavailability of DTX could be increased to as high as 44.3%²⁰³.

When a prodrug approach is unlikely, lymphatic transport *via* particulates creates new opportunities for facilitated oral absorption of drug entities with severe first-pass metabolism. Emulsions consisting of fatty acids of different chain lengths were designed to deliver quercetin²⁰⁴. Significantly increased lymphatic exposure was observed for quercetin and its active metabolites, especially by long-chain fatty acid-based nanoemulsions²⁰⁴. Nitrendipine was delivered as SLNs using three different lipid matrices—tripalmitin, cetyl palmitate and glyceryl monostearate²⁰⁵. All formulations displayed increased oral bioavailability by 3.09–3.93-fold as compared with the suspension counterpart²⁰⁵. By constructing berberine-loaded cremochylomicrons—particles composed of Cremophor EL and Tween 80 to imitate CM, two times higher absorption than the pure drug was detected²⁰⁶. After treating with cycloheximide, a reduction of approximately 43% in oral absorption was observed²⁰⁶, which stands for the same amount of lymphatic transport. More examples with carvedilol and olanzapine prove the partial contribution of lymphatic transport to enhanced oral bioavailability^{174,207}. Inorganic carriers were also exploited with high drug loading and lymphatic targeting property. In a study on triglyceride-mimetic 1,3-dipalmitoyl glycerol-decorated silica nanocarriers, the C_{max} and AUC of the model drug lopinavir were increased by 1.69- and 5.97-fold as compared with free lopinavir²⁰⁸. Pre-treatment with cyclohexamide reduced the plasma C_{max} and AUC by 62.12% and 85.98%, respectively, highlighting the role of lymphatic transport²⁰⁸.

6.2. Facilitated oral absorption by particulates

Lymphatic transport of particulates can be also utilized to enhance the oral absorption of labile or poorly permeable entities such as biomacromolecules, extraordinarily lipophilic drugs, and BCS III and IV drugs. Probuco, with a lgP of 10.3, is a model drug of choice for the study of lymphatic transport⁷³. The oral absorption of probuco is extremely limited because of not only its poor solubility but also retention in enterocytes that line the apical surfaces of microvilli. Formulating probuco into SNEDDS enhances lymphatic transport and overall oral bioavailability by 10.22-fold as compared with probuco suspension^{209,230}. Similarly, formulating baicalin into nanoemulsions promotes its AUC by 14.56-fold, and CM-based lymphatic transport contributes 26.8% to the systemic exposure of the drug as observed in a CM blocking model by using cycloheximide²¹⁰. The oral bioavailability of a SNEDDS formulation of Solvent Green 3, a poorly water-soluble drug was 1.7-fold higher than the soybean oil emulsion, while nearly no absorption was observed after pre-treatment with another CM blocker, colchicine²¹¹. On the other hand, surface modification with polymers or ligands sometimes results in altered oral absorption and transport behaviors. *N*-carboxymethyl chitosan-coated SLNs showed more than 4-fold and near 2-fold increase in lymphatic transport of curcumin in comparison with curcumin solution and uncoated SLNs, respectively, echoing the capacity of opening tight junction and lymphatic transport *via* the paracellular pathway²¹². It is acknowledged that besides improved dissolution and permeability, lymphatic transport also plays a role in enhanced oral absorption of several successfully marketed lipid-based products^{69,213,214,231}. Multiple mechanisms, though difficult to clarify, may act concurrently in lymphatic transport. For instance, not only the CM but also the M cell pathway participates in lymphatic transport of morin phospholipid complex-loaded SNEDDS, as visualized by fluorescence bioimaging after labeling the vehicles with a near infrared fluorescent dye, Nile red²¹⁵. The enhanced lymphatic transport of lutein by a solid dispersion and SMEDDS formulation in different ratios is ascribed to the particles size effect and different transport mechanisms²¹⁶.

As for biomacromolecules like insulin or poorly permeable drugs with virtually no oral absorption, particulate-

facilitated lymphatic transport seems to be more attractive. Furthermore, the relatively high availability of particulates—for example, approximately 13.21% for PCL/Eudragit RS50/50 nanoparticles—makes oral delivery of the most challengeable drug entities possible²¹⁷. In a study on oral delivery of insulin by thermosensitive gel-thickened glucan microparticles²¹⁸, cumulative lymphatic transport was determined by mesenteric lymphatic cannulation together with visualization by live imaging to reveal the translocation of the particles. The pharmacological bioavailability of insulin could reach 9%–10%, and positive correlation was established between mesenteric accumulation and pharmacological bioavailability, which highlighted the contribution of M cell-based lymphatic transport²¹⁸. Another study with insulin-loaded liposomes decorated by chondroitin sulfate-taurocholic acid confirmed the role of the CM pathway in lymphatic transport²¹⁹. After binding with apical sodium-dependent bile acid transporter and being internalized intact, nanoparticles could be transported into the lymph, as evidenced by cannulation and visualized by transmission electron microscopy (TEM)²¹⁹. Similarly, a hydrophilic peptide exenatide was loaded in phase-changeable nanoemulsions that exhibit effective lymphatic transport and higher pancreas accumulation. Evidence collected by both confocal laser scanning microscopy and cycloheximide inhibition helps identify the contribution of the M cell-based lymphatic transport pathway²²⁰.

6.3. Distant site targeting

In view of probable oral absorption of intact particulates as discussed above, peroral targeting of particulates together with payloads to distant sites beyond the gastrointestinal tract is highly expectable. After uptake by M cells, particulates that reach the lymphatic system will be transported from the right lymphatic duct to the subclavian vein and pulmonary artery successively, and finally reach different MPS organs and tissues including the liver, spleen, lungs and kidneys, as well as tumors and inflammatory sites²³². A pioneer study on yeast whole glucan particles revealed apparent accumulation of the particles in organs and tissues, which are abundant in macrophages, ranging from Peyer's patches to spleen, lymph nodes and marrow²³³. Similar results with curdlan, a large particulate β -1,3-glucan, also supports oral absorption of glucan particles and subsequent targeting to distant sites²³⁴.

Distant targeting through the lymphatic pathway for therapeutic purposes has already been reported. Orally delivered WGA-conjugated SLNs loading paclitaxel tend to accumulate in the lung, with 6.2-fold higher local drug levels than pure paclitaxel solution²²¹. By tracking the signals of the fluorescent dye Cy7.5, orally administered YCW microcapsules could be found migrating from intestinal Peyer's patches to mesenteric lymph nodes and then to MPS organs and tissues and finally reaching human lung carcinoma xenografts in mice²²². Chitosan-binding peptide-decorated PLGA nanoparticles loading rifampicin/gantrez attained more than 10 times of drug levels in lung tissues and realized effective antifungal treatment^{171,235}. Although the underlying transport mechanisms are unclear, the scavenging capacity of monocytes in Peyer's patches may play an essential role in particle transport¹⁵¹. The lung, as the first location where lymph originated from the mesentery converges with blood, is the first site for particle retention²³². After leaving the lung, the particles enter the systemic circulation and distribute in other MPS organs such as

the liver achieving high targeting efficiency, as evidenced by sugar-conjugated sorafenib nanoparticles for the treatment of hepatocellular carcinoma²²³. Orally administered particles are able to reach distant inflammatory sites or lesions such as in atherosclerotic vessels^{131,236} and even in the brain as well^{224,237}.

Though the efficiency of peroral targeting is very low in comparison with the intravenous route, multiple dosing may be employed to attain therapeutic-level accumulation in specific organs or tissues²³⁸.

6.4. Treatment involving the immune system

As oral delivery *via* lymphatic transport takes advantages of the intestinal and systemic immune system, it is reasonable to propose that oral lymphatic delivery may be better suited for oral immunization and treatment of diseases inflicting the immune systems. With respect to particle delivery, the protective mechanisms of the human body block the entry of a majority of particulates exposed in the GIT. The limited population of M cells imposes certain challenges for efficient drug delivery²³⁹.

Oral vaccination is the most widely investigated area for particle delivery. Compared to systemic vaccination, oral mucosal vaccination is able to induce both systemic and mucosal immunity and is more preferable than the parenteral routes in terms of safety and convenience in administration^{240–242}. However, the harsh gastrointestinal conditions and presence of multiple barriers limit the efficiency of oral immunization²⁴³. Therefore, researches resort to particulate delivery systems and make use of the immunogenic capacity of follicles in Peyer's patches²⁴⁴. Enhanced and more balanced systemic Th1 and Th2 responses were elicited following oral administration of ovalbumin-loaded polyanhydride nanoparticles coated with mannose and flagellin²²⁵. The high levels of intestinal IgA titers elicited demonstrate enhanced mucosal immune response. It is also worth noting that a balance between Th1 and Th2 is beneficial to immune responses owing to the Th1/Th2 mutual adjustment effect^{245,246}. Although this study did not give detailed information about drug transport, the possible contribution of M cell was highlighted. In another study on oral immunization with hepatitis B surface antigen (HBsAg) by utilizing lectin-conjugated PLGA nanoparticles, higher levels of serum anti-HBsAg antibodies and mucosa-secreted IgA were detected and efficient M cell uptake was recorded by confocal laser scanning microscopy²²⁶. Consecutive dosing of HBsAg-loaded liposomes decorated with UEA 1 for 3 days elicits maximum serum levels of anti-HBsAg IgG comparable to muscular delivery after three weeks²²⁷. In addition, mucosal IgA secreted locally is able to migrate to systemic mucosal systems, thus possibly enabling distant mucosal immunity *via* oral delivery²⁴⁷. The vaccines may be released from the vehicles after uptake¹⁶⁵, but some particles such as glucan microparticles may remain intact in macrophages for a long time. Exploration of particle behaviors *in vivo* is essential in understanding the underlying mechanisms of immunization.

Besides oral vaccination, lymphatic drug delivery is beneficial for the treatment of diseases inflicting the immune systems. Conjugation of mycophenolic acid (MPA), an immunomodulator, to triglycerides (2-MPA-TG) enhances the drug concentration in lymphocytes by 103-fold and MPA level in lymphatic nodes by 28-fold²²⁸. The bexarotene and retinoic acid carboxylic ester prodrug produces similar enhanced drug levels in lymphocytes by 17-fold and lymph nodes by 2.4-fold²²⁹. Although no

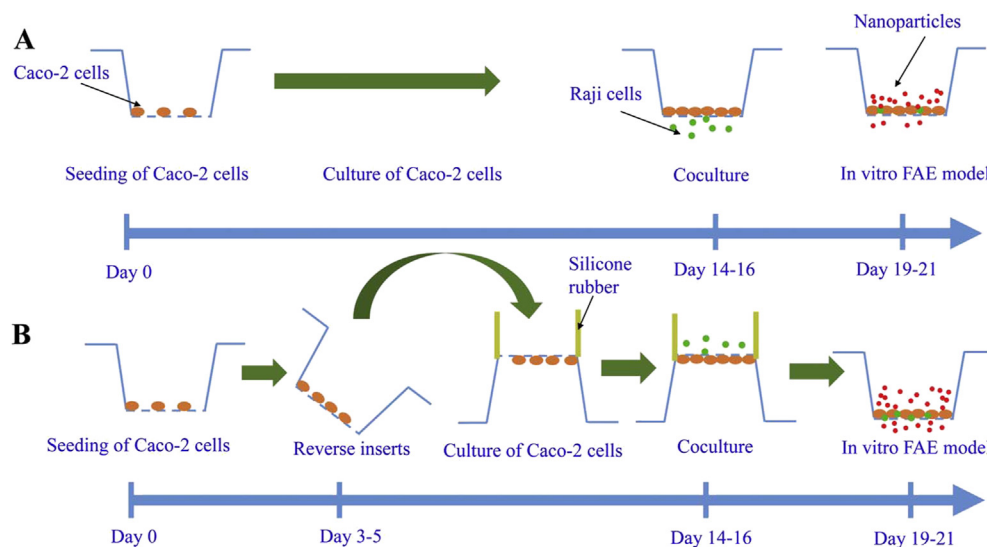


Figure 4 Schematic of *in vitro* M cell-like model. (A) "Non-inverted" method; (B) "Inverted" method. Reprinted with the permission from Ref. 253. Copyright © 2007 Elsevier.

pharmacological results are reported for these two studies, the lymphatic drug enrichment is believed to benefit the efficacy of treatment.

7. Models for evaluation of lymphatic transport

Several models have been developed for the evaluation of lymphatic transport. The *in vitro* models aim at mimicking the function of either M cells or CMs, while the *in vivo* models mainly aim at collection and estimation of lymphatic drug transport in total *via* lymphatic cannulation.

7.1. *In vitro* model

7.1.1. M-like cell model

Caco-2 cell lines have been established as an *in vitro* model for the evaluation of drug permeation efficiency by cellular uptake and transmonolayer transport experiments. Addition of lymphocytes imparts Caco-2 cell lines (monolayers) with M cell-mimicking capacities¹²⁶. This is commonly done by co-culturing Caco-2 cells with lymphoid cells like Raji cells or murine derived lymphocytes^{248,249}. Among different culture protocols, filter-grown co-culture Caco-2/Raji system was the most investigated one²⁵⁰. The first proposed model was practiced by seeding Caco-2 cells on the inverted insert and incubating with lymphoid cells on the downside of the insert²⁵¹. However, this method suffers from multiple variations and low reproducibility²⁵⁰. Hence, modified methods, namely the "non-inverted" and "inverted" method, have been developed^{252,253}. In a "non-inverted" model, Raji cells are added directly to the basolateral side of an insert, while in an "inverted" model, Raji cells are added after inverting the insert (Fig. 4)^{23,253}. In a common M-like model, the lymphoid cells are allowed to permeate through the gaps on the filters and co-culture with Caco-2 cells without altering the function of each cell lines²⁵¹. The "inverted" model has higher reproducibility and more robustness, though more difficult to conduct than the "non-converted" one²⁵⁰. Successful M cell transformation is usually verified by observation of increased attachment of particles such as bacteria or viruses, altered expression of certain genes, changed morphology with irregular microvilli by TEM and scanning electron microscopy^{44,254,255}. M-

like models have been applied to study of basic physiology, selection of ligands and simulation of pathogen transport^{234,248,256}. In recent years, the models have been well adapted for prediction of enteric uptake of biomacromolecules and particulates^{160,257,258}. Nevertheless, *in vitro* models should not be utilized to replace *in vivo* models because of the simplified cell monolayer structures and presence of excessively high content of M-like cells which may lead to overestimation of experimental results²⁵⁹.

7.1.2. Chylomicron association model

As virtual association of drug molecules with CMs in enterocytes governs lymphatic drug transport efficiency, *in vitro* CM association model may provide crucial information for the prediction of *in vivo* mesenteric transport. DDT with greater CM affinity showed significantly higher lymphatic transport compared to diazepam with limited binding efficiency⁸³. Linear correlation between CM binding capability and lymphatic transport was established with nine high fat-soluble components including halofantrine, probucol, vitamins, etc⁷¹. Physicochemical properties concerning this behavior were further testified by an *in silico* model⁶⁸. Although natural CMs may work better in prediction, biomimetic CMs are preferred due to lower inter-group variations, reduced number of animals sacrificed and avoidance of tedious operations for separation of natural CMs⁷³. The insignificant difference observed between natural and biomimetic CM models justifies the utilization of the latter for drug association study²⁴⁷. In addition to basic physicochemical properties of drugs, a series of factors such as addition of surfactants may also matter due to possible alternation of drug solubility⁷⁴. Therefore, the contribution of excipients should also be considered.

7.2. *In vivo* model

7.2.1. Lymphatic cannulation model

As lacteals that collect particles or drug-associated CMs absorbed from the apical side coverage at the mesenteric lymphatics, mesenteric lymphatic cannulation is a good option to collect and measure total lymphatic drug transport. Therefore, the mesenteric lymphatic cannulation model has been established and utilized for

evaluation of lymphatic transport^{79,260,261}. Detailed surgery procedures can be found in literature²⁶². The drug content in the collected lymphatic fluid can be quantified by assaying measures such as HPLC and LC-MS/MS, whereas the content of particles can be quantified based on specific physicochemical signals associated with the particles or constituting materials^{263–265}. Big animals (pig, dog) are preferred to smaller ones (rat and mouse) because of physiological and anatomical keenness (*e.g.*, biliary secretion) to human^{266,267}. Moreover, it is easy to simulate pre- and post-prandial states in big animals than in small ones which display constant bile flow regardless of dietary⁸¹. Nevertheless, large animals are more costly for preliminary drug screening and more difficult to procure and handle. Taking together all factors, the rat model is the most popular model available. Thoracic cannulation is an alternative option reported with operational convenience, especially in mice²⁶⁸, but it may be less accurate in estimation of lymphatic transport because the thoracic lymphatic duct collects lymph from lymphatics other than the mesenteric lymphatic duct⁴².

Despite the easiness associated with sample collection and quantification, lymphatic cannulation is invasive and thus associated with certain limitations²⁶⁹. Cannulation involves complex surgery and may alter the lymph flow and vessel pressure gradient, which make consecutive sampling difficult after several sampling treatments. Owing to the influence of multiple factors, the success rate of lymphatic cannulation is generally very low^{263,270}. Therefore, further improvements or substitutes are highly expected.

7.2.2. Lymph blocking model

In order to avoid operational stress involved in cannulation, lymph blocking models have been established by utilizing blocking reagents. Cycloheximide, a protein synthesis inhibitor, selectively suppresses the secretion of CMs but does not affect other absorption pathways. Though the blockage is irreversible, no obvious adverse effects have been recorded in literature²⁷¹. The high correlation of the CM blocking model with lymphatic cannulation model validates its reliability for the prediction of lymphatic absorption^{272,273}. However, whether cycloheximide specifically blocks CMs remains arguable. Researches indicate that cycloheximide may have inhibitory effect on the M cells too, which thus compromises the estimation accuracy of the CM pathway²⁷⁴. Other inhibitors with different blocking mechanisms such as Pluronic L-81, diacylglycerol acyl transferase inhibitor and colchicine have also been reported²⁶³. Similarly, the specificity of these blocking agents is yet to be tested too.

Several M cell blocking models have been utilized to assess the contribution of the M cells. NF- κ B ligand (RANKL) expressed on the epithelium is essential for M cell differentiation. After treatment with anti-RANKL antibody, oral pathogenesis study was successfully done²⁷⁵. B cell knocking is another model established to characterize M cell depletion²⁷⁶. The pathogen translocation mechanism is identified involving both Peyer's patches and epithelia. Furthermore, recombination-activating gene 2 (Rag 2) and gamma chain (γ c) deficient BALB/c mice (Rag- γ c^{-/-}) were also applied to investigate oral norovirus infection²⁷⁷. The lack of Peyer's patches and mature M cells demonstrates successful establishment of the models. Though numerous studies have showed the feasibility of M cell deficient model for disease investigation, there are still no research reported in drug absorption evaluation.

8. Conclusions and perspectives

Intestinal lymphatic transport is emerging as an attractive strategy for oral drug delivery with precious advantages that other strategies cannot accommodate, such as evading first-pass metabolism, enhancing overall oral bioavailability, and benefiting treatment of lymphatic diseases. By now, there have been fundamentally two pathways confirmed for intestinal lymphatic transport—that is, the chylomicron and M cell pathways. Though there are reports that transcellular and paracellular penetration across enterocytes may be involved, the potential contribution of these pathways is yet to be proved. The chylomicron pathway takes advantage of the lipolysis and absorption mechanisms of fat or oil which is primarily comprised of triglycerides. Both triglyceride-mimetic and long-chain fatty acid prodrugs are able to achieve greatly enhanced oral bioavailability with the contribution of lymphatic transport being as high as more than a half of the original dose. The M cell pathway presents as the main portal in the GIT for the entry of particulates into the body. However, the efficiency of this pathway is restricted to less than approximately 10% in general, probably owing to the limited population of M cells in enteric epithelia and the retention in sub-FAE follicles but limited escape and transport to the lymphatics. For better evaluation of lymphatic transport efficiency, several *in vitro* and *in vivo* models have been established for different lymphatic transport pathways, though none of them provide adequate information to verify the actual amount transported *via* the lymphatics.

Lymphatic delivery provides an alternative option for drug discovery and development, especially for those with poor stability, low solubility and permeability. With appropriate molecular and/or formulation designs, therapeutic levels of active pharmaceutical ingredients can be attained in both lymphatic and systemic circulation by exploiting enteric lymphatic transport. Currently, several relevant drug products have been marketed with sound therapeutic effects as well as commercial success. In the near future, more lymphatic transport-oriented products are foreseeable in view of the ever-growing challenges and financial burden associated with conventional roadmap of drug discovery. On the other hand, there are as well various unsolved problems involved in lymphatic drug delivery despite the promises. The influencing factors have not been fully unraveled and there is a lack of effective quantification measures and both *in vitro* and *in vivo* models for accurate estimation of the contribution of lymphatic transport, thus hindering the product and clinical translation greatly. However, it is envisioned that with the advancement of technologies like bioimaging and a better knowledge of the *in vivo* behaviors of drug molecules and the delivery vehicles, lymphatic transport efficiency can be enhanced substantially to fulfill not only therapeutic but also unidentified commitment such as targeting to remote sites beyond the GIT.

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Author contributions

Zichen Zhang wrote the manuscript. Wei Wu, Zicen Zhang, Yi Lu and Jianping Qi revised the manuscript. All of the authors have read and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

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