Intracellular transport of nanocarriers across the intestinal epithelium

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The intestinal epithelium is the main barrier restricting the oral delivery of low-permeability drugs. Over recent years, numerous nanocarriers have been designed to improve the efficiency of oral drug delivery. However, the intracellular processes determining the transport of nanocarriers across the intestinal epithelium remain elusive, and only limited enhancement of the oral bioavailability of drugs has been achieved. Here, we review the processes involved in nanocarrier trafficking across the intestinal epithelium, including apical endocytosis, intracellular transport, and basolateral exocytosis. Understanding the complex intracellular processes of nanocarrier trafficking is particularly essential for the rational design of oral drug delivery systems.

Introduction

Oral administration is the preferred route of drug delivery. However, the intestinal epithelium is the main barrier to the oral delivery of poorly permeable drugs. For oral delivery, drugs must cross the intestinal epithelium before reaching the blood circulation. Unfortunately, there are many low-permeability drugs, commonly classified as biopharmaceutical classification system III and IV drugs, that are unable to diffuse across the intestinal epithelium [1–3]. The intestinal epithelium comprises mainly polarized epithelial cells. During polarization, epithelial cells develop biochemically and functionally distinct apical and basolateral membrane domains that are separated by tight junctions.

To facilitate oral delivery, low-permeability drugs are usually encapsulated in nanocarriers, such as micelles, liposomes, and polymer nanoparticles (NPs), which have physicochemical properties that enable them to overcome the intestinal epithelial barrier [4,5]. Although numerous nanocarriers have been applied for oral delivery over the past two decades, the mechanisms behind their intracellular transport across the epithelial cell barrier remain elusive.

Nanocarriers must undergo the following steps before crossing the intestinal epithelium: endocytosis at the apical side; transport through the cytoplasm; and exocytosis at the basolateral side [6]. Nanocarriers are internalized into cells through various pathways, such as clathrin-mediated endocytosis, lipid raft-mediated endocytosis, and macropinocytosis. After being endocytosed, nanocarriers interact with different organelles, including endosomes, lysosomes, endoplasmic reticulum (ER), and the Golgi apparatus, and utilize different transport routes, such as the endolysosomal, ER/Golgi, and cytoplasmic routes, leading to distinct destinations. Whether these interactions of nanocarriers with organelles are beneficial to the transcellular delivery of drugs remains to be investigated.

Here, we consider the entire trafficking process of nanocarriers across the intestinal epithelium (Fig. 1), with a focus on the intracellular transport of nanocarriers. The process is divided into three parts: Step 1, apical endocytosis of nanocarriers; Step 2, intracellular transport of nanocarriers; and Step 3, basolateral exocytosis of drugs. We emphasize that the conclusions and discussions of the three parts are mostly based on the results from studies in cells rather than *in vivo*. In addition, we also discuss the methods used to study the intracellular transport of nanocarriers, including the use of pharmacological inhibitors and optical microscopy (Table 1). Understanding the complex intracellular process of nanocarriers is essential for the rational design of oral drug delivery systems.

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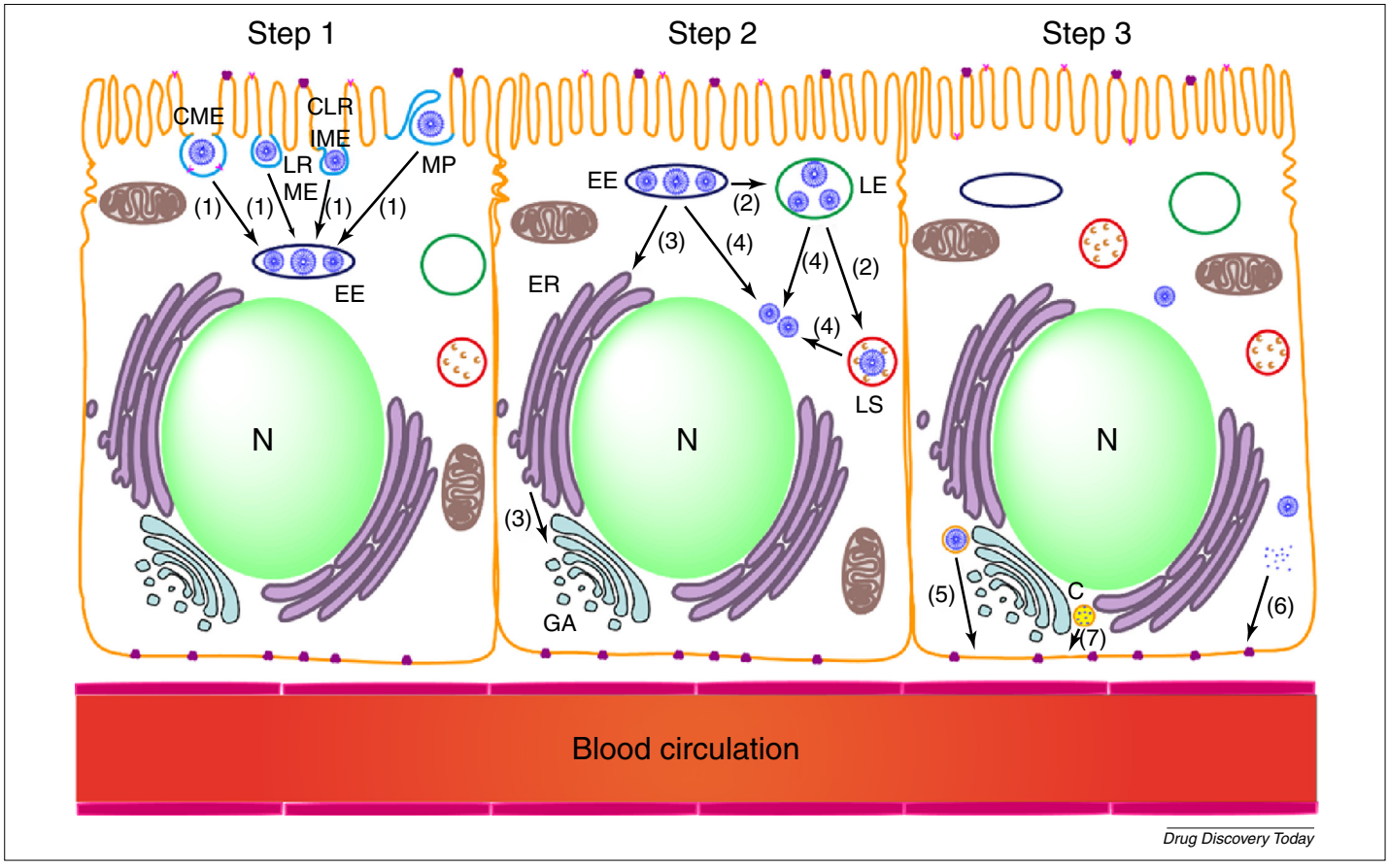

FIGURE 1

Schematic illustration of the trafficking process of nanocarriers. Step 1. Apical endocytosis of nanocarriers. Step 2. Intracellular transport of nanocarriers. Step 3. Basolateral exocytosis of drugs. (1) Various types of apical endocytosis; (2) endolysosomal route; (3) endoplasmic reticulum (ER) and Golgi apparatus (GA) route; (4) endolysosomal escape and cytoplasmic route; (5) vesicle-mediated exocytosis; (6) transporter-mediated exocytosis; (7) chylomicron (C)-mediated exocytosis. *Abbreviations*: CLRIME, clathrin- and lipid raft-independent endocytosis; CME, clathrin-mediated endocytosis; EE, early endosomes; LE, late endosomes; LRME, lipid raft-mediated endocytosis; LS, lysosomes; MP, macropinocytosis; N, nucleus.

Step 1. Apical endocytosis of nanocarriers

The apical membrane of the small intestinal enterocytes that underlie the mucus layer is the first barrier restricting the transcellular delivery of nanocarriers. Given that nanocarriers as large as 10–1000 nm cannot diffuse passively across the membrane, apical endocytosis appears to be important for cell entry. Initially, nanocarriers must interact with proteins embedded in the apical membrane or associate directly with the lipid bilayer for cellular internalization to occur. Receptors are the most frequently utilized apical proteins to enhance the cellular endocytosis of nanocarriers. In addition to receptors, there are some negatively charged glycoproteins and proteoglycans in the apical membrane with which nanocarriers can nonspecifically interact via hydrophobic and electrostatic interactions [7].

Receptor-mediated endocytosis

Over the past decade, extensive efforts have been devoted to designing active-targeted drug delivery systems (ATDDS) that utilize receptor-mediated endocytosis, and are now considered to be promising for oral drug delivery [8]. In ATDDS, nanocarriers are often functionally modified with ligands that bind to receptors with high affinity and specificity. Representative ATDDS currently under development for oral delivery, and their *in vitro* and *in vivo* enhancement ratios, are summarized in Table 2.

The transferrin (TfR), vitamin B_{12} (VB12), and immunoglobulin G (IgG; FcRn) receptors are the most widely explored receptors involved in ATDDS. Designing functional nanocarriers that can actively target these receptors and be internalized through receptor-mediated endocytosis, has the potential to significantly enhance the cellular uptake of nanocarriers, thus leading to increased intracellular drug concentrations.

It was recently reported that the cellular internalization of polymeric NPs with exterior targeting TfR proteins was approximately fivefold higher than that of bovine serum albumin (BSA)-coated NPs. Likewise, insulin-loaded Tf-coated NPs induced more significant hypoglycemic effects [9]. TfR is involved in the uptake of transferrin and iron from the intestinal lumen. It has the advantage of being rapidly recycled back to the cell membrane, enabling the quick internalization of nanocarriers targeted to this receptor. However, despite the high expression of TfR on the intestinal epithelium, this receptor is distributed predominantly on the basolateral surface [10].

Previous reports showed that VB12-modified dextran NPs with different insulin-loading capacity showed more profound and

TABLE 1

Methods used to study the intracellular transport of nanocarriers[a].

Pharmacological inhibition			Optical microscopy	
Inhibitors	**Functions**	**Mechanisms**	**Markers**	**Organelles**
Chlorpromazine	Inhibits clathrin-mediated pathway	Rho GTPase inhibition	EEA1	Early endosomes
Filipin	Inhibits lipid raft-mediated pathway	Interacting with cholesterol	Rab5	Early endosomes
Nystatin	Inhibits lipid raft-mediated pathway	Interacting with cholesterol	Rab7	Late endosomes
MβCD/lovastatin	Inhibits clathrin and lipid raft-independent pathway	Cholesterol depletion	Lysotracker	Endosomes and lysosomes
Amiloride	Inhibits macropinocytosis	Lowering submembraneous pH and preventing Rac1 and Cdc42 signaling	LAMP1	Lysosomes
Cytochalasin D	Inhibits macropinocytosis	Inhibiting actin polymerization	ER tracker	ER
Bafilomycin A1	Inhibits endosomal acidification	Inhibiting vacuolar type H^+ ATPases	TGN46	Trans-Golgi network
Brefeldin A	Inhibits ER/Golgi pathway	Triggering retrograde transport of Golgi enzymes back to ER	Golgi tracker	Golgi apparatus
Monensin	Inhibits Golgi/PM pathway	Inhibiting transportation of macromolecules from Golgi complex to PM		

[a] *Abbreviation*: MβCD, methyl-β-cyclodextrin.

prolonged hypoglycemic effect than nontargeted NPs [11,12]. VB12 is an essential nutrient and will bind to intrinsic factor (IF) before being endocytosed by the intestinal epithelium through a receptor-mediated pathway. The VB12 receptor is highly expressed on the apical membrane of ileum enterocytes, but only recognizes the IF-VB12 complex, rather than IF or VB12 alone [13,14]. Disappointingly, the limited distribution of the VB12 receptor in the ileum hinders the efficient endocytosis of nanocarriers actively targeted to this receptor.

Pridgen *et al*. revealed that transepithelial transport of Fc-targeted NPs was twofold greater than nontargeted NPs. Moreover, by calculating the total ^{14}C in all of the organs, the oral absorption of ^{14}C-labeled Fc-targeted NPs was 11.5-fold higher than nontargeted NPs [15]. FcRn is expressed at the apical surface of neonatal epithelial cells in the proximal small intestine, where it binds specifically to IgG and transports across the intestinal epithelium, releasing IgG at the basolateral side into the blood. The pH difference between the apical and basolateral sides of intestinal epithelial cells facilitates the efficient unidirectional transport of IgG because FcRn binds IgG at pH 6.0–6.5, but releases it at higher pH values [16].

In addition to the ATDDS mentioned above, there are several other active-targeted nanocarriers. It was reported that folic acid-functionalized NPs were able to target folate receptors to improve the oral delivery of paclitaxel. Cellular uptake studies showed that the intracellular accumulation of paclitaxel was 1.5-fold higher for

TABLE 2

Summary of ATDDS for oral delivery and the enhancement ratio *in vitro* and *in vivo* compared with unmodified nanocarriers[a].

Receptor	Drug	Nanocarrier	Material	Size (nm)	Zeta potential (mV)	Cellular uptake enhancement ratio (fold)	P_{app} enhancement ratio (fold)	Bioavailability enhancement ratio (fold)[b,c]	Refs
Biotin receptor	Insulin	Liposomes	SPC, DSPE, and CH	150	NS	1.69 (1.5 h)	1.85 (4 h)	2.52	[67]
FcRn	Insulin	NPs	PLA-*b*-PEG	63	−5.6 ± 1.1	NS	2 (24 h)	11.5[c]	[15]
Folic acid receptor	Insulin	Liposomes	PC, SA, CH, PAA, and PAH	266.2 ± 10.4	+25.4 ± 2.6	3.33 (3 h)	NS	1.28	[68]
Integrin receptor	Insulin	NPs	PLGA-mPEG	~200	~+16	1.84 (3 h)	1.90 (3 h)	1.42	[69]
Lectin receptor	Calcitonin	Liposomes	DSPC, SA, and CH	191.8	−41.9 ± 4.8	2 (2 h)	NS	3	[17]
TfR	Coumarin 6	Micelles	PEG-*b*-PCL	35.94 ± 2.76	−3.1 ± 0.84	1.44 (10 min); 1.52 (1 h)	2.3 (0.5 h); 1.8 (2 h)	NS	[35]
VB12 receptor	Insulin	NPs	Dextran	192	NS	NS	NS	1.1 (2%, w/w); 1.9 (3%, w/w); 2.6 (4%, w/w)	[11]

[a] *Abbreviations*: CH, cholesterol; DSPC, L-α-distearoylphosphatidylcholine; DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphatidyl ethanolamine; NS, not studied; PAA, poly (acrylic acid); PAH, poly (allylamine hydrochloride); P_{app}: apparent permeability coefficient; PC, phosphatidylcholine; PEG-*b*-PCL, poly (ethylene glycol)-block-poly (ε-caprolactone); PLA-*b*-PEG, poly (lactide acid)-*b*-poly (ethylene glycol); PLGA-mPEG, poly (lactide-co-glycolide)-monomethoxy-poly (ethylene glycol); SPC, soybean phosphatidylcholine; SA, stearylamine.
[b] w/w, the drug loading capacity.
[c] Calculated by the total absorbed ^{14}C in all of the organs.

folic acid-modified NPs than for the free drug over a 2-h period [5]. Moreover, wheat germ agglutinin (WGA)-modified liposomes significantly enhanced the cellular uptake of coumarin 6 compared with non-modified liposomes. In addition, the uptake was dependent on the surface WGA concentration, temperature, and incubation period [17].

Pinocytosis of nanocarriers

Types of pinocytosis

Both receptor-mediated endocytosis and nonspecific endocytosis by the intestinal epithelium are forms of pinocytosis. The molecular mechanisms of pinocytosis include clathrin-mediated endocytosis, lipid raft-mediated endocytosis, clathrin- and lipid raft-independent endocytosis, and macropinocytosis [18]. Although caveolae-mediated endocytosis is mentioned in various publications, there is some controversy over whether there are caveolae present on the apical membrane of the intestinal epithelium. Caveolae were first identified as an endocytic compartment in endothelial cells [19]. However, many researchers believe that Caco-2 cells or enterocytes have no caveolae on the apical membrane [20–23]. Thus, here we consider lipid raft-mediated endocytosis instead of caveolae-mediated endocytosis.

Abramov *et al.* showed that monomethoxy polyethylene glycol–polylactic acid (mPEG–PLA) micelles were endocytosed via various pathways except macropinocytosis. Interestingly, small interfering (si)RNA knockout of caveolin genes in Caco-2 cells did not inhibit the endocytosis of mPEG-PLA micelles. This finding suggested that there are no caveolae on the apical membrane of enterocytes [24]. Endocytic mechanisms of nanocarriers can also be investigated through inhibiting certain pathways by using pharmacological inhibitors and then detecting the amounts taken up (Table 1). The detailed mechanisms as well as the advantages and disadvantages of pharmacological inhibitors are reviewed elsewhere [25].

Effect of the properties of nanocarriers on pinocytosis

Nanocarriers are often internalized into cells via various pathways. The physicochemical properties of nanocarriers have a significant effect on endocytosis pathways and the uptake amounts.

Effect of size

When nanocarriers are less than 200 nm in diameter, size might have no effect on endocytosis pathways. NPs made from soy proteins with a similar negative surface charge but different sizes (30, 100, and 180 nm) showed similar endocytosis mechanisms. The cellular uptake of these NPs was via clathrin- and/or lipid raft-mediated endocytosis and macropinocytosis, with 100-nm NPs exhibiting the greatest uptake [26].

Effect of surface charge

Nanocarriers with opposite surface charges have different cellular entry routes. For positively charged polystyrene NPs, clathrin-mediated endocytosis, clathrin- and lipid raft-independent endocytosis, and macropinocytosis are involved in cellular internalization. By contrast, negatively charged NPs are endocytosed through a lipid raft-mediated pathway. In general, positively charged NPs yield a higher level of internalization [27]. However, as an oral drug delivery system, the effect of the mucus layer should be taken into consideration, because the positively charged surface hinders the effective mucus penetration of nanocarriers as a result of nanocarriers adhering to the negatively charged mucin. In this case, nanocarriers with a positively charged core and dissociable 'mucus-inert' hydrophilic coating can be an excellent choice [28].

Effect of surface modification

The surface modification of nanocarriers can affect the cellular endocytosis mechanisms. As reported previously, lipid raft-mediated endocytosis was involved in the uptake of Pluronic F127-adsorbed liposomes by Caco-2 cells, which was the same as that for unmodified liposomes. Compared with Pluronic F127-adsorbed liposomes, clathrin- and lipid raft-mediated endocytosis were both involved in the cellular uptake of Pluronic F127-inlaid liposomes [29].

Effect of ligands

Transporters are another type of functional protein embedded in the cell membrane, in addition to receptors. Nanocarriers modified with specific ligands can be targeted to transporters and, thus, improve interactions with the cell membrane that can enhance the cellular uptake. There are many highly expressed transporters distributed in the apical membrane of the intestinal epithelium, such as the apical sodium-dependent bile acid transporter (ASBT) and proton-coupled folate transporter. Folate-modified poly (lactic-co-glycolic acid) (PLGA) copolymer NPs could significantly improve the oral bioavailability of insulin compared with normal NPs [30]. Furthermore, the high affinity-binding macromolecules functionally transformed the ASBT so that ASBT–macromolecule complexes were internalized in vesicles in a process similar to receptor-mediated endocytosis [31].

Effect of hardness

Although there are few articles discussing the effect of hardness on the cellular endocytosis of nanocarriers, several *in vitro* and *in silico* studies have demonstrated that this is an important factor. Dissipative particle dynamics simulation studies showed that rigid NPs enter the cell more readily by endocytosis, whereas this process is more limiting for soft NPs [32]. Cellular studies have consistently confirmed that significantly higher uptake of the more rigid PLGA–lipid NPs is achieved compared with the less rigid PLGA–water–lipid NPs [33].

Generally, more rigid nanocarriers of a suitable size, and with a positive surface charge and functional ligands targeting specific receptors and transporters will result in more endocytosis [34]. Thus, the rational design of nanocarriers can effectively improve their cellular uptake. However, many researchers suggest that the amount of drugs that are transported account for only a small fraction of the total drugs added [35,36]. Therefore, there must be intracellular barriers in addition to the apical membrane that limit the transcellular delivery of nanocarriers.

Step 2. Intracellular transport of nanocarriers

Endolysosomal route

Regardless of the cellular endocytosis pathway used, nanocarriers are encapsulated in vesicles and then trafficked into the endosome. Endosomal trafficking is a complicated process involving shuttling of the nanocarriers along the microtubules within the cell and the endosomes maturing into lysosomes [7]. Under normal circumstances, nanocarriers are transported from endosomes to lysosomes

via the endolysosomal route. Surface charges do not alter the endolysosomal route of nanocarriers during nonspecific internalization. Both cationic and anionic poly(amidoamine) dendrimers were observed to be trafficked from early endosomes to lysosomes through immunofluorescent staining of early endosome antigen 1 (EEA1) and lysosome-associated membrane protein 1 (LAMP1) [37]. In addition to the nonspecific internalization of nanocarriers, receptor-mediated endocytosis of ligand-modified nanocarriers involves a similar endolysosomal route.

Du *et al*. developed functional nanocarriers (7pep-M-C6) that actively targeted TfR for receptor-mediated endocytosis. A quantitative colocalization analysis demonstrated that both M-C6 and 7pep-M-C6 accumulated in early and late endosomes. The authors also observed that the colocalization of 7pep-M-C6 with LysoTracker® started as early as 5 min after incubation [35]. However, LysoTracker is not specific for lysosomes and will stain any acidic organelle, including endosomes [38]. Therefore, the fast colocalization seen with LysoTracker only confirmed the quick internalization of 7pep-M-C6.

Disadvantages of the endolysosomal route

Early endosomes receive endocytosed cargos through a series of internalization pathways as described above, and will mature into late endosomes and then into lysosomes with rapid acidification from pH 6.5 to 5.0 [39,40]. Lysosomal pH is maintained at approximately 5.0 by a proton-pumping ATPase in the lysosomal membrane. There are many enzymes inside the lysosome that are most active at an acidic pH [41]. Cargo transported into lysosomes, such as receptors, signaling proteins, polysaccharides, and lipids, is degraded by these enzymes. The degradation products, including amino acids, glucose, and fatty acids, are released into the cytoplasm to meet the nutritional needs of the cell [42]. Thus, the entrapment of nanocarriers within lysosomes is undesirable for the transcellular delivery of drugs that are designed to cross the intestinal epithelium.

Intracellular transport studies utilizing WGA-functionalized NPs (WGA-NPs) with various polymer architectures confirmed that WGA-NPs with shorter surface PEG lengths resulted in more colocalization via the clathrin-mediated transport pathway. By contrast, WGA-NPs with longer surface PEG lengths avoided the clathrin-mediated transport pathway. Given that the clathrin-mediated pathway targets lysosomes [43], formulations with longer surface PEG lengths achieved higher transcytosis. Furthermore, WGA-NPs with PLGA as the core material exhibited improved lysosomal escape and enhanced transcytosis compared with WGA-NPs with a PLA core [44].

ER and Golgi routes

The retrograde trafficking pathway is an alternative route of intracellular transport that avoids the acidic and hydrolytic lysosomal environment. This pathway leads nanocarriers inside the endosomes to the ER and Golgi apparatus. Some toxins, such as the shiga and cholera toxins, exploit the retrograde trafficking pathway to localize in the ER and interfere with its function [45]. The retrograde trafficking pathway is also involved in recycling certain receptors, such as the mannose-6 phosphate receptor [42,46]. Although no studies have reported the rational design of nanocarriers to take advantage of the retrograde trafficking pathway for oral delivery, nanocarriers could engage with the ER and Golgi routes by chance.

Gao *et al*. developed quantum dot-loaded WGA-NPs to track cellular transport pathways. WGA-NPs were partially transported to the Golgi apparatus. Importantly, when microtubules were depolymerized by nocodazole, and actin polymerization was disrupted by cytochalasin D, the colocalization of WGA-NPs with the Golgi apparatus increased. These results suggest that the accumulation of WGA-NPs in the Golgi apparatus resulted mainly from the blocking of post-Golgi trafficking by inhibiting the cytoskeleton [47].

Cell-penetrating peptide modifications are widely used to improve the internalization of nanocarriers. In studies by Fujiwara *et al*., liposomes were modified by the octaarginine (R8) cell-penetrating peptide. R8-modified liposomes were first found to be trapped in the endosomal compartment. Later, a fraction of the liposomes were observed to colocalize with the Golgi apparatus. Similar consequences were also visualized for liposomes modified with another cationic peptide, octalysine (K8). However, cationic liposomes containing 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) colocalized predominantly with lysosomes and not with the Golgi apparatus. Therefore, a small portion of liposomes modified with cell-penetrating peptides could be transported to the Golgi apparatus, possibly via a retrograde pathway after being endocytosed [48].

Advantages of the ER and Golgi routes

The ER and Golgi apparatus are vital components of the secretory ER/Golgi and endocytic recycling pathways [49]. Nanocarriers are believed to be transported out of a cell when arriving at the Golgi apparatus because this apparatus is involved in the transport of macromolecules to the plasma membrane (PM). However, more studies are needed to clarify how to utilize the secretory ER/Golgi pathway efficiently. It is possible that some nanocarriers, based on toxins and equipped with similar physicochemical properties, would be able to escape the lysosomal compartment via the retrograde pathway and further improve the basolateral exocytosis of nanocarriers.

Cytoplasmic route: escape from endosomes and lysosomes

Lysosomal enzymes can be detrimental to the integrity of nanocarriers and the bioactivity of drugs. Therefore, it is desirable to escape endolysosomes and move into the cytoplasm. In most instances, nanocarriers enter the intestinal epithelium through various endocytosis pathways, become entrapped in the endolysosomal compartments, and are further degraded by abundant hydrolytic enzymes in the lysosomes. Thus, a limiting step in achieving effective drug therapy, especially for siRNA drugs, is to facilitate their endosomal escape and overcome lysosomal degradation [50].

Several strategies have been proposed to facilitate the endolysosomal escape of nanocarriers. These involve pH-responsive nanocarriers that disperse drugs into the cytosol from endolysosomes, and endolysosome-disrupting agents that aid in the release of nanocarriers into the cytosol [51]. Typically, endolysosomal escape occurs through pore formation, rupture, or membrane fusion by the nanocarriers [52]. A large amount of research has been reported in the area of parenteral gene delivery. Endolysosomal escape is required for gene delivery because the target site of a gene is inside the cytoplasm [53–55]. However, there are few nanocarriers available for oral drug delivery, even for drugs such as proteins and peptides that are vulnerable to lysosomal enzymes. The low amount of drugs transported across the intestinal epithelium can result from lysosomal degradation. Thus, lysosomal degradation could be an intracellular barrier for the oral delivery

of proteins and peptides, although more research is required to overcome this barrier.

The cytoplasm is not the preferred destination for transcellular transport of nanocarriers because many drugs need to enter the blood circulation to exert their pharmacological activity. If nanocarriers or drugs are unable to exit cells from the basolateral side, cytoplasmic delivery might be unfavorable. Therefore, more studies are needed to determine whether the cytoplasmic route will improve the transcytosis of nanocarriers.

Step 3. Basolateral exocytosis of drugs

In addition to apical endocytosis and intracellular trafficking of nanocarriers, it is important to ensure the basolateral exocytosis of drugs to improve their oral bioavailability. However, there are few articles discussing the direct basolateral exocytosis of nanocarriers, although several studies have verified the existence of nanocarriers in the basolateral medium of Caco-2 cell monolayers by using transmission electron microscopy (TEM) [35,56].

Vesicle-mediated exocytosis

Exocytosis is often defined specifically as the upward transport of endocytosed drugs across the apical PM. Vesicle-mediated exocytosis of drugs often involves a series of organelles, especially the ER and Golgi apparatus, which are important regulators of the secretory ER/Golgi pathway. Pharmacological inhibitors are used to study the effect of some specific pathways, organelles, and proteins on the exocytosis of drugs (Table 1). For example, brefeldin A can block the ER/Golgi pathway by triggering the retrograde transport of Golgi enzymes into the ER [57]. Monensin mainly inhibits the transport of cargo from the Golgi complex to the PM as a result of disruption of the Golgi complex [58].

He *et al*. studied the exocytosis mechanism of PLGA polymer NPs (PNs) in Caco-2 cells. They found that both the ER/Golgi and Golgi/PM pathways were involved in the exocytosis of PNs. Additionally, they found that disrupting lipid rafts increased the exocytosis of PNs by promoting fusion of recycled endosome components with the PM. However, quantitative and qualitative studies showed that the cellular uptake of PNs in Caco-2 cells was significant, whereas transcytosis was not. This supported the concept of 'easy entry and hard across' for PNs [59]. Moreover, a study of solid lipid nanocarrier (SLN) exocytosis in MDCK cells in the presence of brefeldin A and monensin also demonstrated the positive role of the ER and Golgi complex. By contrast, inhibiting the ER/Golgi and Golgi/PM pathways increased the transcytosis of SLNs across the entire transport process. Chai *et al*. hypothesized that there might be direct pathways that transport SLNs to the basolateral side and, thus, inhibitors of the ER and Golgi-mediated pathways could improve the activity of these direct pathways [60]. However, basolateral exocytosis can differ from apical exocytosis as a result of the distinct protein distribution of the polarized intestinal epithelium. Therefore, basolateral exocytosis might be reduced if apical exocytosis is strong.

Transporter-mediated exocytosis

In addition to vesicle-mediated exocytosis, nanocarriers can release drugs into the cytoplasm to avoid lysosomal degradation. In this situation, drugs can be designed to exit the cells through the basolateral transporters. The known transporters expressed in the basolateral membrane of the intestinal epithelium that can transport specific substrates into the blood circulation include the organic solute transporter and multidrug resistance-associated proteins 1 (MRP1) and MRP3 [61]. The exocytosis of free drugs will be increased if they are capable of combining with the basolateral transporters. The vectorial transport of fexofenadine across Caco-2 cells was reported to take advantage of the basolateral transporter MRP3 [62].

Chylomicron-mediated exocytosis

Chylomicrons, which comprise a hydrophobic core and a hydrophilic surface, are assembled by triglycerides inside the intestinal epithelium. Chylomicrons fuse with the basolateral cell membrane and are released into the interstitial space. After basolateral exocytosis, chylomicrons are transported selectively into lymphatic capillaries [63]. Therefore, lipophilic drugs might have access to the lymphatic system through association with chylomicrons [64]. Chylomicrons might also promote the intestinal absorption of lipopolysaccharides because of the high affinity between chylomicrons and lipopolysaccharides [65]. In addition, docetaxel nanocapsules were observed inside the abdominal mesenteric lymph nodes by cryogenic temperature TEM after oral delivery. The lymphatic absorption of docetaxel nanocapsules was facilitated by chylomicron-mediated exocytosis [66].

Given that endocytosis can be improved through many strategies, basolateral exocytosis often restricts the transcellular delivery of drugs. There are currently only a few strategies for enhancing the basolateral exocytosis of drugs or nanocarriers, demonstrating a need to increase research in this area.

Concluding remarks

The intestinal epithelium is believed to be the main physiological barrier to the oral delivery of low-permeability drugs. As a promising drug delivery system to conquer this biological barrier, nanocarriers have the potential for apical endocytosis, intracellular transport, and basolateral exocytosis. NPs with optimized physicochemical properties, such as positive surface charge and high-affinity surface ligands, can improve apical endocytosis. To avoid lysosomal degradation and improve intracellular diffusion, the retrograde pathway might be the best strategy for nanocarriers, although more studies are needed to investigate how to manipulate it. Likewise, functional nanocarriers with endolysosomal escape capability will be helpful. Finally, biomimetic strategies can be utilized to enhance the basolateral exocytosis. However, the integrity of nanocarriers and mechanisms behind the release of drugs from them are still unclear and more research is required in this area. Thus, a better understanding of the intracellular transport of nanocarriers and related cellular functions will shed more light on the optimized design of nanocarriers to meet the demand for their medical application.

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