

# Advanced drug delivery to the lymphatic system: lipid-based nanoformulations

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**Abstract:** The delivery of drugs and bioactive compounds via the lymphatic system is complex and dependent on the physiological uniqueness of the system. The lymphatic route plays an important role in transporting extracellular fluid to maintain homeostasis and in transferring immune cells to injury sites, and is able to avoid first-pass metabolism, thus acting as a bypass route for compounds with lower bioavailability, ie, those undergoing more hepatic metabolism. The lymphatic route also provides an option for the delivery of therapeutic molecules, such as drugs to treat cancer and human immunodeficiency virus, which can travel through the lymphatic system. Lymphatic imaging is useful in evaluating disease states and treatment plans for progressive diseases of the lymph system. Novel lipid-based nanoformulations, such as solid lipid nanoparticles and nanostructured lipid carriers, have unique characteristics that make them promising candidates for lymphatic delivery. These formulations are superior to colloidal carrier systems because they have controlled release properties and provide better chemical stability for drug molecules. However, multiple factors regulate the lymphatic delivery of drugs. Prior to lymphatic uptake, lipid-based nanoformulations are required to undergo interstitial hindrance that modulates drug delivery. Therefore, uptake and distribution of lipid-based nanoformulations by the lymphatic system depends on factors such as particle size, surface charge, molecular weight, and hydrophobicity. Types of lipid and concentration of the emulsifier are also important factors affecting drug delivery via the lymphatic system. All of these factors can cause changes in intermolecular interactions between the lipid nanoparticle matrix and the incorporated drug, which in turn affects uptake of drug into the lymphatic system. Two lipid-based nanoformulations, ie, solid lipid nanoparticles and nanostructured lipid carriers, have been administered via multiple routes (subcutaneous, pulmonary, and intestinal) for targeting of the lymphatic system. This paper provides a detailed review of novel lipid-based nanoformulations and their lymphatic delivery via different routes, as well as the in vivo and in vitro models used to study drug transport in the lymphatic system. Physicochemical properties that influence lymphatic delivery as well as the advantages of lipid-based nanoformulations for lymphatic delivery are also discussed.

**Keywords:** lymphatic system, blood circulation, solid lipid nanoparticles, nanostructured lipid carriers

## Introduction

Over the past 25 years, vascular research has primarily focused on the biology of blood rather than the biology of lymph because of the difficulties involved in visualizing the lymphatic system and a lack of appreciation of its distinctive function. Currently, the lymphatic system is gaining more interest and achieving more recognition outside of cancer biology.

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The lymphatic system is part of the circulatory system and is comprised of an intricate network of conduits that carry a clear fluid called lymph. The primary functions of the lymphatic system are to maintain the body's water balance by returning extracellular fluid that has leaked out into the interstitial space back to the systemic circulation and to transport immune cells to the lymph nodes.<sup>1,2</sup> Further, the lymphatic system has specialized roles in specific areas because of its nonuniform structure and function throughout the body. It plays an essential role in absorption of long-chain fatty acids, triglycerides, cholesterol esters, lipid soluble vitamins, and xenobiotics.<sup>2,3</sup> Drug delivery via the lymphatic system has several major advantages, including circumventing first-pass metabolism in the liver and targeting drugs to diseases that spread through the lymphatic system (eg, certain types of cancer and human immunodeficiency virus). The lymphatic system also plays an active role in disseminating metastatic cancer cells and infectious agents throughout the body. Cancer cells use the lymph nodes as a reservoir to spread to other areas of the body.<sup>2,4-7</sup>

There are three ways to deliver drugs through the intestinal lymphatic vessels.<sup>8,9</sup> First, lymphatic capillaries are comprised of single-layered, nonfenestrated endothelial cells. These cells are arranged in a highly gapped and overlapped manner to form a porous wall in the lymphatic vasculature, which allows for macromolecular targeting to the lymphatic system.<sup>3</sup> Therefore, increased absorption of hydrophilic macromolecules and macroconjugates is possible by opening up the paracellular route with the help of an absorption enhancer.<sup>10</sup> Secondly, gut-associated lymphoid tissue consists of either isolated or aggregated lymphoid follicles that form Peyer's patches, which provide an entry point for drug to the lymphatics (Figure 1A).<sup>11-14</sup> Finally, the primary route for lipid transport is through the intestinal walls via transcellular absorption, paracellular transport, P-glycoprotein, and cytochrome P450 inhibition. Increased production of chylomicrons is associated with delivery of lipophilic compounds into the lymphatic system (Figure 1B).<sup>9</sup> Utilization of this route is discussed in this review in terms of lymphatic targeting of lipid-based nanoformulations.

A number of lipid-based formulations, including emulsions, micellar systems, self-emulsifying drug delivery systems, self-microemulsifying drug delivery systems, self-nanoemulsifying drug delivery systems, liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) have been investigated as drug carriers for the lymphatic system (Table 1).<sup>15-46</sup>

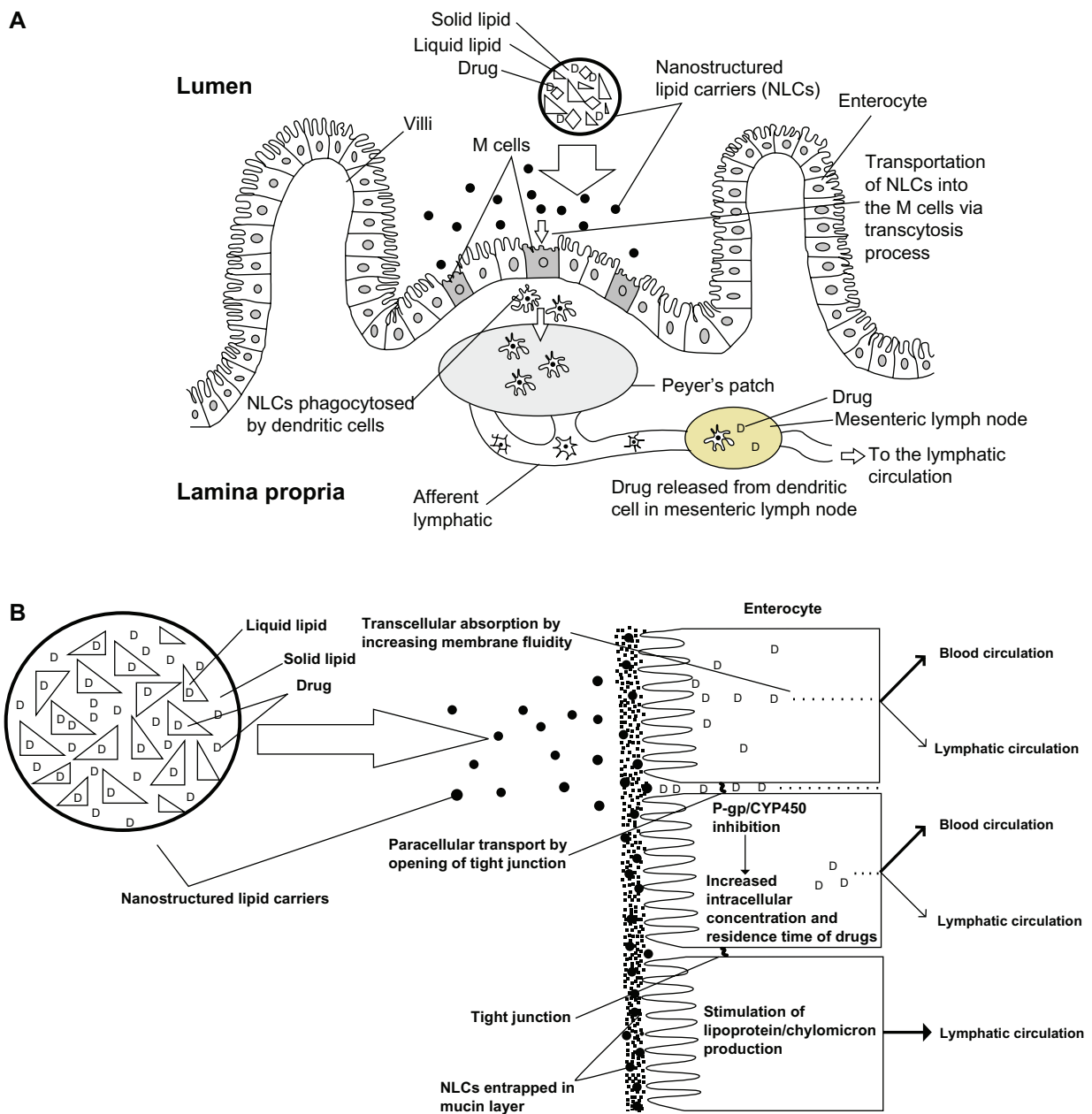
Some therapeutic agents have also been incorporated into polymer-based lipid nanoparticles to improve lymphatic drug delivery. Using this approach, the anatomy of the lymphatic system determines delivery of the therapeutic agent, ie, the architecture of the endothelial wall of the lymphatic vessel provides the open space necessary to facilitate delivery of a complex of high molecular weight drug polymers. These studies were conducted using anticancer molecules to potentiate lymphatic delivery. A number of natural (dextran and hyaluronic acid) and synthetic (polyhexylcyanoacrylate, polymethylmethacrylate, poly[L-lactic acid], and poly[lactic-co-glycolic acid]) polymers have been used as carriers to deliver drugs through the lymphatic system.<sup>47-54</sup>

The lymphatic system was previously thought to play a passive role in the spread of disease throughout the body; however, recent findings have opened up a new chapter regarding the role of the lymphatic system in the metastasis of cancer. The discovery of specific markers and growth factors in the lymphatic endothelium, such as vascular endothelial growth factor (VEGF)-C, VEGF-D, and VEGF-A, the VEGF-D receptor (VEGFR-3), and Prox-1, have provided the opportunity for specific drug targeting to diminish lymphangiogenesis and metastasis in the lymphatic system.<sup>55</sup>

In the development of drug delivery to target progressive lymphatic disease, such as human malignancy, lymphatic imaging techniques play a crucial role in planning treatment. The disease invades the lymphatic system in the first stage of progression during metastasis. Lymphatic imaging techniques can be used to evaluate both the disease state and the effectiveness of drug therapy. Imaging techniques using visible dyes and radionuclides do not produce clear images; however, new techniques such as fluorescence imaging, magnetic resonance imaging, quantum dots, and nanocarriers have been shown to have greater sensitivity and higher resolution, while eliminating unnecessary biopsies or removal of healthy nodal tissue.<sup>56-59</sup>

## Lipid-based nanoparticles

Lipid-based nanoparticles containing a solid matrix are generally divided into two groups, ie, SLNs and NLCs. In the early 1990s, SLNs were identified as an alternative to colloidal drug carriers, such as liposomes, microemulsions, nanoemulsions, and nanocapsules.<sup>60</sup> Both SLNs and NLCs have many advantages compared with other colloidal carrier systems, including controlled drug release and improved chemical stability of drug molecules. Moreover, these carrier systems can also be produced on a large scale.<sup>61-63</sup>



**Figure 1** (A) Schematic transverse section of Peyer's patch, illustrating M cell transportation of lipid based formulation (nanostructured lipid carriers) to the lymphatic vessels. (B) Schematic diagram of the different mechanisms of the intestinal transport of lipid based formulation (nanostructured lipid carriers) through blood and lymphatic circulation. **Abbreviations:** P-gp, P-glycoprotein; CYP450, cytochrome P450 enzyme; M cell, membranous cell.

## Solid lipid nanoparticles

SLNs offer a prominent advantage over other nanoparticulate systems because they use physiological lipids and surfactants, which are generally recognized as safe. The commonly used lipids in the SLNs preparation are fatty acids, waxes, monoglycerides, diglycerides, and triglycerides; surfactants such as poloxamer and polysorbate are also widely used. Further, the possibility of avoiding a solvent using high-pressure homogenization can help avoid the carrier biotoxicity problem in humans.<sup>64,65</sup> SLNs involve formation

of a relatively rigid core consisting of lipids that are solid at room temperature. Thus, SLNs can help improve stability and provide controlled release and drug targeting.<sup>66</sup> The minute size of this formulation enables efficient uptake of drugs into the intestine, particularly via the lymphatic route, involving particles only 20–500 nm in diameter.<sup>67</sup>

Absorption via the lymphatic route can be used for delivery of cytotoxic agents to overcome the limitations of nonspecificity, drug resistance, and severe toxicity.<sup>68</sup> Several cytotoxic drugs have been incorporated into SLNs, including

**Table 1** Formulations that have been used for lymphatic targeting

| Formulations     | Drugs                | References |
|------------------|----------------------|------------|
| Emulsion         | Penclomedine         | 15         |
| Emulsion         | Ontazolast           | 16         |
| Microemulsion    | Puerarin             | 17         |
| Microemulsion    | Raloxifene           | 18         |
| Micellar systems | Cyclosporin A        | 19         |
| SEEDS            | Coenzyme Q10         | 20         |
| SMEDDS           | Halofantrine         | 21         |
| SMEDDS           | Nobiletin            | 22         |
| SMEDDS           | Valsartan            | 23         |
| SMEDDS           | Vinpocetine          | 24         |
| SMEDDS           | Silymarin            | 25         |
| SMEDDS           | Sirrolimus           | 26         |
| SNEDDS           | Carvedilol           | 27         |
| SNEDDS           | Valsartan            | 28         |
| SNEDDS           | Halofantrine         | 29         |
| Liposomes        | IgG1                 | 30         |
| Liposomes        | Doxorubicin          | 31–33      |
| Liposomes        | Cefotaxime           | 34         |
| Liposomes        | 9-nitro-camptothecin | 35         |
| Liposomes        | Paclitaxel           | 36         |
| Liposomes        | Ovalbumin            | 37         |
| SLNs             | Etoposide            | 38         |
| SLNs             | Methotrexate         | 39         |
| SLNs             | Idarubicin           | 40         |
| SLNs             | Tobramycin           | 41,42      |
| SLNs             | Nimodipine           | 43         |
| NLCs             | Testosterone         | 44         |
| NLCs             | Vinpocetine          | 45         |
| NLCs             | Triptetine           | 46         |

**Abbreviations:** SEEDS, self-emulsifying drug delivery systems; SMEDDS, self-microemulsifying drug delivery systems; SNEDDS, self-nanoemulsifying drug delivery systems; SLNs, solid lipid nanoparticles; NLCs, nanostructured lipid carriers.

idarubicin, methotrexate, and etoposide.<sup>38–40,69</sup> However, conventional intravenous administration of cytotoxic drugs has limited tumor uptake because of minimal access to the tumor, decreased circulation time due to faster clearance by the phagocytic system, and decreased targeting.<sup>70</sup> Therefore, alternative routes of administration have been explored for SLNs, including the subcutaneous, pulmonary, and duodenal routes.<sup>38–42,69,71–73</sup>

## Subcutaneous route for lymphatic delivery of SLNs

A study comparing the various routes of administration for etoposide-loaded tripalmitin (ETPL) in SLNs was performed by Harivardhan et al in mice bearing Dalton's lymphoma.<sup>38</sup> This study compared the biodistribution of radiolabeled free etoposide and radiolabeled ETPL nanoparticles via three different administration routes, ie, intravenous, subcutaneous, and intraperitoneal. Etoposide and ETPL

nanoparticles were labeled using <sup>99m</sup>Tc (Technetium) and detected using a gamma ray spectrometer and gamma scintigraphy. Subcutaneous administration showed superior tumor uptake at 24 hours compared with both intraperitoneal and intravenous administration, with 8-fold higher drug uptake than intraperitoneal and 59-fold higher drug uptake than intravenous routes. Subcutaneous administration also showed a significant reduction in drug uptake by organs of the reticuloendothelial system (ie, lung, liver, and spleen), which resulted in longer circulation of ETPL nanoparticles. This route also had a relatively low tissue distribution, which can reduce the systemic side effects of etoposide. Initial uptake of ETPL nanoparticles by the tumor after subcutaneous administration was low, but increased over time. This slow deposition of ETPL nanoparticles suggests the possibility for controlled release therapy. Thus, subcutaneous injection of drug adjacent to the tumor site could be a better route for chemotherapeutic treatment of lymphatic-related tumors than intravenous or intraperitoneal administration.<sup>38</sup>

## Pulmonary route for lymphatic delivery of SLNs

Targeted delivery of SLNs via the pulmonary route has significant potential in certain types of cancer. Some solid endocrine tumors, such as small cell lung carcinoma, show high levels of metastatic proliferation. These tumors spread initially through one hemithorax and its regional lymph nodes, eventually travelling through the lymphatic system to the blood circulation.<sup>74–77</sup> This type of cancer metastasis relies on drainage from the lymph nodes.<sup>74,76</sup> Further, alveolar clearance of drug particles up to a certain diameter (200 nm) involves the lymphatic system.<sup>71,78,79</sup> This renders drug targeting with SLNs feasible, and several studies have been done involving delivery of SLNs in patients with lung cancer via nebulization and gene therapy.<sup>72,80</sup>

Videira et al formulated an SLNs system incorporating paclitaxel, which is widely used in the treatment of non-small cell lung cancer.<sup>72</sup> In that study, nebulization of paclitaxel-loaded SLNs was compared with intravenous administration of paclitaxel alone using a conventional formulation in mice inoculated with MXT-B2 cells to develop lung metastases. Treatment with paclitaxel-loaded SLNs demonstrated a significant 20-fold reduction in inhibitory concentration of 50% of cell growth (IC<sub>50</sub>) values and a 19.43% reduction in cell viability compared with intravenous administration of paclitaxel alone. Unlike intravenous paclitaxel, the SLNs formulation showed an absence of toxicity with prolonged treatment,

suggesting that SLNs delivery has high selectivity and low systemic circulation. Uptake of SLNs into the lymphatic system was demonstrated earlier by Videira et al using radiolabeled SLNs whereas other researchers used a computed tomography contrast agent.<sup>71,79</sup> Both studies demonstrated that biodistribution of SLNs occurs primarily via the lymphatic system rather than by the blood circulation. Thus, specific delivery of cytotoxic drugs via inhalation using SLNs could be a promising option for chemotherapy in the future.

## Intestinal route for lymphatic delivery of SLNs

The gastrointestinal tract is the preferred route for drug delivery. However, because of its unique anatomy and physiology, several factors may affect drug bioavailability, including the drug's solubility in the gastrointestinal tract, the pH in the tract, and the amount of time spent there.<sup>73</sup> This route also subjects drugs to presystemic hepatic metabolism, which can reduce drug bioavailability. To overcome this, the lymphatic absorption of SLNs can be exploited by incorporating drugs into SLNs to circumvent first-pass metabolism. Several groups of researchers have explored this and reported increased bioavailability when SLNs incorporating drugs are administered intraduodenally.<sup>39-41</sup>

The superior uptake of methotrexate through the lymphatic system and into the systemic circulation has been demonstrated in methotrexate-loaded SLNs.<sup>39</sup> In this study, the effect of different types of lipid-based SLNs was investigated using stearic acid, monostearin, tristearin, and Compritol® 888 ATO. Intraduodenal administration of methotrexate-loaded SLNs showed increased bioavailability of methotrexate regardless of the types of lipid used, with the greatest increase observed in SLNs containing Compritol 888 ATO compared with the methotrexate solution. A 10-fold increase in methotrexate concentration was observed in the lymphatic system with methotrexate-loaded SLNs compared with the methotrexate solution.

Another study incorporated idarubicin into SLNs and compared this with an idarubicin solution for intraduodenal and intravenous administration.<sup>40</sup> Duodenal administration of idarubicin-loaded SLNs enhanced drug bioavailability, as indicated by a 21-fold increase in the area under the curve compared with the idarubicin solution. This study also showed less distribution of idarubicin to the heart, lung, spleen, and kidneys, which may reduce the cardiotoxicity of idarubicin. Because the elimination half-life of idarubicin-loaded SLNs was increased by 30-fold compared with idarubicin solution,

it was suggested that SLNs could be useful as a prolonged-release system. The study also showed a higher area under the curve when idarubicin-loaded SLNs were administered intraduodenally compared with intravenous administration of the same formulation. These findings show that drugs formulated with SLNs can provide specific targeted drug delivery to increase clinical efficacy and reduce the toxicity of oral anticancer agents.<sup>40</sup>

## Nanostructured lipid carriers

The lipid-based NLCs system was developed to overcome the limitations of SLNs, such as drug loading into a solid matrix and drug expulsion during storage because of polymorphic modification of the lipid particles. SLNs use only one form of lipid, ie, a solid lipid that orients the drug between the fatty acid chains of glycerides. In contrast, NLCs use a blend of both solid and liquid lipids to form a controlled nanostructure. Imperfections between the lipids provide spaces to accommodate the drugs in the matrix, resulting in maximum drug-loading capacity.<sup>81,82</sup> Further, NLCs are less susceptible than SLNs to gelation during both preparation and storage.<sup>81,83</sup> Thus, NLCs are considered to represent a second generation of lipid nanoparticle formulations.<sup>81</sup>

## Subcutaneous route for lymphatic delivery of NLCs

The subcutaneous route is an attractive one for lymphatic delivery of lipid nanoparticles, with several advantages, including drug accumulation at the site of administration for a longer period of time, low clearance, sustained release, and increased absorption. On subcutaneous administration, lipid nanoparticles are not directly transported into the bloodstream because capillaries control the permeability of water and small molecules. Instead, the lymphatic capillaries surrounding the subcutaneous injection site absorb the lipid-based nanoparticles. Absorption of these lipid-based nanoparticles into the lymphatic system depends primarily on the size of the nanoparticles. Larger lipid nanoparticles accumulate at the injection site, and the drug is slowly released from the nanoparticles. The free drug can enter the blood circulation via pores on the walls of the capillaries. Smaller lipid nanoparticles (<0.1 μm) can easily access the lymphatic capillaries and concentrate in regional lymph nodes.<sup>84</sup> Thus, based on these advantages, NLCs could be developed as a carrier for lymphatic drug delivery by subcutaneous administration because they have improved physicochemical properties compared with other lipid-based nanocarrier systems.

## Pulmonary route for lymphatic delivery of NLCs

Drug administration via the pulmonary route has several advantages compared with the oral and parenteral routes. The pulmonary route avoids first-pass metabolism, reduces systemic toxicity, is noninvasive, minimizes the need for continuous dosing, allows the drugs administered to reach less accessible parts of the lung directly, and enables increased local concentrations of drug.<sup>85</sup> The pulmonary route shows great potential for the delivery of NLCs into the lymphatic circulation. The particle size of NLCs can be reduced to less than 500 nm, which could increase drug deposition in the lung epithelium because of their diffusional mobility.<sup>86</sup> NLCs are lipid-based nanoparticles that could be used as a carrier for targeting drugs to small cell lung cancer and human immunodeficiency virus, both of which spread through the lymphatic system and can cross into the systemic circulation.<sup>4-7,87</sup> Thus, NLCs have the potential to provide a drug delivery mechanism via the lymphatic system through the pulmonary route and may have increased effectiveness compared with SLNs.

## Intestinal route for lymphatic delivery of NLCs

NLCs have the potential to be an effective method for oral drug delivery, because they can increase solubility and enhance the oral bioavailability of drugs that are either hydrophobic or poorly soluble in water.<sup>88</sup> Among the traditional lipid-based formulations, NLCs have become an important alternative to the more traditional colloidal drug carriers.<sup>81</sup> Zhuang et al developed drug-loaded NLCs to improve the oral bioavailability of vinpocetine.<sup>45</sup> Both vinpocetine-loaded NLCs and a vinpocetine suspension were orally administered to male Wistar rats. The time taken to reach maximum plasma concentrations ( $T_{max}$ ) and the peak concentration reached ( $C_{max}$ ) for the vinpocetine suspension were 30 minutes and  $354.29 \pm 57.49$  ng/mL, respectively, whereas the  $T_{max}$  and  $C_{max}$  of vinpocetine-loaded NLCs were 1.5 hours and  $679.29 \pm 135.57$  ng/mL, respectively. The  $T_{max}$  for vinpocetine-loaded NLCs was one hour longer than for the vinpocetine suspension, indicating indirect transport of NLCs into the systemic circulation. The  $C_{max}$  for vinpocetine-loaded NLCs was also significantly higher than for the vinpocetine suspension. The area under the curve for the vinpocetine-loaded NLCs was 3.2-fold greater than that of the vinpocetine suspension. In vivo pharmacokinetic analysis showed a 322% increase in the relative bioavailability of vinpocetine-loaded NLCs compared with the vinpocetine

suspension after oral administration. These results suggest that NLCs can improve the oral bioavailability of drugs which are poorly soluble in water.<sup>45</sup> One possible reason for the enhanced bioavailability of vinpocetine could be that NLCs are transported in the lymphatic system, so largely avoid first-pass metabolism, which is the main cause for the low bioavailability of vinpocetine.<sup>89,90</sup>

In another study, Zhou et al developed tripterine NLCs and evaluated their potential as an oral drug delivery system.<sup>46</sup> A rat intestinal perfusion model was used to compare the absorption of tripterine-loaded NLCs with that of a tripterine solution. The effective permeability of tripterine NLCs in the duodenum, jejunum, ileum, and colon was 2.1, 2.7, 1.1, and 1.2 times higher, respectively, compared with the tripterine solution. The percentage absorption of tripterine-loaded NLCs in 10 cm of duodenum, jejunum, ileum, and colon was 2.2, 2.3, 1.2, and 1.3 times greater, respectively, than for the tripterine solution.<sup>46</sup> These results indicate that NLCs could be used as a carrier to improve the absorption of tripterine in the gastrointestinal tract.

## Models used to study drug transport in the lymphatic system

### In vivo models

In the in vivo model, cannulation of the mesenteric or thoracic lymphatic ducts is performed in animals to investigate drug transport in the intestinal lymphatic system.<sup>91</sup> This model allows for direct measurement of drug concentrations in lymph. Because it is an irreversible and invasive surgical process, the procedure cannot be performed on humans.<sup>92</sup> Small animals, such as rats, are commonly used, but some larger animals, including sheep, pigs, rabbits, and dogs, have also been used for this model.<sup>92-100</sup>

Another in vivo model is the lymphatic venous shunt, in which drug concentrations in lymph are measured at fixed time intervals, and lymph is collected over a longer period of time. Further, an indirect method has been used in an oral bioavailability study to evaluate intestinal lymphatic drug transport in both the presence and absence of inhibitors of intestinal chylomicron flow. This method has the advantage of not requiring a surgical procedure, as does the lymphatic duct cannulation model.<sup>101-104</sup>

### In vitro models

Various in vitro models can serve as an alternative to in vivo models for studying lymphatic drug transport. In the intestinal permeability model, Caco-2 cells are used to evaluate intracellular lipoprotein-lipid assembly

and to examine the effect of lipids and lipidic excipients on incorporation of drug with lipoproteins in lymphatic transport.<sup>88,105–107</sup> In one *in vitro* model, Gershkovich and Hoffman described a correlation between the degree of *ex vivo* incorporation of a drug into chylomicrons and the extent of intestinal lymphatic drug transport.<sup>108</sup> According to a lipolysis model described by Dahan and Hoffman, *in vivo* drug absorption could be predicted by evaluating drug release from a lipid-based drug delivery system and estimating precipitation of the drug during lipolysis.<sup>109</sup> Holm and Hoest reported an *in silico* method that established a quantitative relationship between the molecular structure and amount of drug transferred from the intestinal to the lymphatic system.<sup>102–104,110</sup>

## Factors affecting transport of nanoparticles to the lymphatic system

Gastrointestinal labile molecules such as anticancer, anti-HIV and immunosuppressant compounds have been incorporated into lipid-based nanocarriers. The uptake and distribution of the lipid-based nanocarriers through the gastrointestinal epithelium to the peripheral lymphatic duct have been explored.<sup>111–113</sup> Some groups have reported that uptake of lipid-based nanoparticles by the lymphatic system and their distribution in the lymphatic circulation is dependent on route of administration. Moreover, other factors such as size, surface charge, molecular weight, hydrophobicity, types of lipid, and concentration of the emulsifier used have also been observed to influence the uptake and distribution of lipid-based nanoparticles in the lymphatic circulation.<sup>12,39,53,69</sup>

### Size of nanoparticles

The size and composition of nanoparticles play an important role in lymphatic uptake and particle retention in lymph nodes. Carriers such as colloidal and lipid particles show more efficiency in lymphatic uptake. Several drug molecules, including anticancer and monoclonal antibodies, have been incorporated into dendrimers and lipid-based nanoparticles, such as liposomes, SLNs, and NLCs, on the basis of their size and the nature of the preparations for lymphatic targeting.<sup>38,114</sup> Oussoren et al reported that a particle size of 10–100 nm is optimal for lymphatic uptake via subcutaneous administration.<sup>115</sup> A particle size smaller than 10 nm is absorbed via the systemic circulation, whereas a particle larger than 100 nm shows preferential uptake via the lymphatic system but at a slower rate. However, particles larger than 100 nm have not been clearly defined.

Further, the authors observed that interstitial injection of particles larger than 100 nm was taken up slowly, and that the particles were trapped at the injection site for a significant period of time.

### Surface charge on nanoparticles

The charge on a drug carrier is also an important factor in lymphatic uptake. Some negatively charged carriers, such as dendrimers, proteins, polylactic-co-glycolic acid nanospheres, and lipid-based nanoparticles (eg, liposomes) have been reported to show higher lymphatic uptake than neutral or positively charged surfaces, which could be due to the fact that the interstitial matrix contains a net negative charge.<sup>116–120</sup> Therefore, in the interstitium, anionic carrier particles encounter electrostatic repulsion and move more quickly.<sup>12,118,121</sup> Highly negatively charged particles have been reported to be retained for a longer period of time in the lymph nodes.<sup>116,117</sup> Conversely, positively charged particles in the interstitium encounter more resistance to move towards the negatively charged interstitium matrix because of the increased electrostatic attraction force. The zeta potential provides information regarding the ionic nature of carrier particles. A zeta potential  $< -30$  mV indicates a strongly anionic nature, values between  $+10$  and  $-10$  mV indicate neutral behavior, and values  $> +30$  mV indicate a cationic nature.<sup>122</sup>

Kaur et al evaluated a zidovudine-loaded liposomes<sup>123</sup> incorporated with either positively (ie, stearylamine) or negatively (ie, dicetyl phosphate) charge surfactants for lymphatic targeting. Using fluorescent microscopy, the organ distribution and lymphatic uptake of these surface-engineered liposomes was determined, and it was reported that the negatively charged liposomes showed improved lymphatic uptake compared with the positively charged liposomes. Another study by Patel et al observed the order of liposomal uptake by the lymph nodes to be as follows: negative  $>$  positive  $>$  neutral charge.<sup>117</sup>

### Molecular weight of drugs

Lymphatic drug delivery via the subcutaneous route shows a linear relationship between molecular weight and extent of absorption of macromolecules. Increasing the molecular weight causes a decrease in uptake of molecules by the capillaries and increased uptake into the lymphatic system at the injection site. Molecules weighing less than 1000 Da are easily absorbed by the capillaries before they are taken into the lymphatic circulation. In contrast, molecules weighing more than 16,000 Da tend to be absorbed by the lymphatic system rather than by the capillaries.<sup>12</sup>

## Hydrophobicity of nanoparticles

Hawley et al showed that hydrophobicity plays an important role in facilitating lymphatic uptake of lipid-based nanoformulations from the administration site.<sup>12</sup> The hydrophobicity of the particles can be correlated with their surface properties, and is mainly responsible for phagocytosis and lymphatic uptake. Dahlback et al demonstrated that decreasing the hydrophobicity of bacteria would decrease phagocytosis.<sup>124</sup> The increased opsonization could be because opsonins attach more easily to hydrophobic surfaces than to hydrophilic surfaces. Because of this phenomenon, phagocytosis would increase, thus increasing lymphatic uptake.<sup>125</sup>

## Lipid solubility and partition coefficient of drugs

Lipid solubility and the partition coefficient are essential physicochemical properties of drugs, and have a major role in lymphatic drug transport. For example, Charman and Stella reported that triglyceride solubility and the log *P* value of a drug should be >50 mg/mL and >5, respectively, for effective lymphatic transport.<sup>126</sup> They compared the lymphatic transport of dichlorodiphenyltrichloroethane and hexachlorobenzene, which have log *P* values of 6.19 and 6.53, respectively. Although the log *P* values of both drugs were similar, the drugs were dissimilar in their triglyceride solubility, with dichlorodiphenyltrichloroethane having a 13-fold higher triglyceride solubility than hexachlorobenzene. Their transport results showed that dichlorodiphenyltrichloroethane had higher lymphatic uptake (33.5%) than hexachlorobenzene (2.3%). These authors concluded that the difference in lymphatic transport could be due to the difference in triglyceride solubility between the two drugs. However, Myers and Stella observed in their study that higher log *P* values and increased lipid solubility did not always result in significant lymphatic uptake.<sup>15</sup> Penclomedine has poor lymphatic transport (only about 3% of the dose administered is transported) despite its log *P* value of 5.48 and lipid solubility of 175 mg/mL. Reduced lymphatic transport of penclomedine could be due to the stronger affinity of this drug for red blood cells and plasma proteins than for chylomicrons. Thus, higher concentrations of penclomedine have been detected in the blood circulation than in the lymphatic circulation.<sup>15</sup>

## Types of lipids used in nanoparticles

Lipid-based nanoformulations are essentially composed of triglycerides which arrange themselves in such a way

that the polar head is exposed to the aqueous phase. This arrangement is similar to that of chylomicrons. The composition of lipids in lipid-based nanoformulations may influence their absorption through the transcellular route via polar intestinal epithelial cells. Paliwal et al prepared methotrexate-loaded SLNs and evaluated the effect of lipids on the characteristics of the formulation.<sup>39</sup> These authors prepared methotrexate-loaded SLNs using the solvent diffusion method with four different types of lipids, ie, Compritol 888 ATO, tristearin, stearic acid, and monostearin. The studied formulations were compared for their size, charge, morphology, drug entrapment, in vitro release, and pharmacokinetic properties. The methotrexate-loaded SLNs containing Compritol 888 ATO had the highest entrapment efficiency and the smallest size compared with the other three types of lipid. The advantages of Compritol 888 ATO over the other lipids could be because of the longer chain length of glyceryl behenate, which provides the interchain insertion site for the methotrexate molecule. These authors observed that methotrexate-Compritol 888 ATO SLNs had better bioavailability than the other methotrexate-loaded SLNs formulations studied. This in situ study evaluated lymphatic uptake using cannulation of the mesenteric duct in an anesthetized albino rat model. The lymphatic drug concentration profile showed that the methotrexate-Compritol 888 ATO SLNs formulation had the highest lymphatic uptake compared with the other methotrexate-loaded SLNs formulations.<sup>39</sup> Further, the authors observed a correlation between their in vitro and in situ results.

## Concentration of emulsifiers in nanoparticles

The concentration of the emulsifier directly influences partitioning of a drug in a lipid-based formulation. Thus, it can indirectly affect delivery of the drug in the lipid-based formulation to the target site. Sanjula et al prepared carvedilol SLNs containing 5%–15% poloxamer 188 as an emulsifier.<sup>127</sup> The authors evaluated the effect of various concentrations of poloxamer 188 on entrapment efficiency and lymphatic uptake. They found that increasing the concentration of the emulsifier would decrease the entrapment efficiency. This could be due to the formation of micelles at higher concentrations of poloxamer 188 causing the solubility of carvedilol in the water phase to increase and leading to lower drug entrapment in the SLNs. The in vivo study was performed with four carvedilol SLNs formulations using varying concentrations of poloxamer 188 administered via the intraduodenal route in male Wistar rats.



Higher area under the concentration-time curve values were measured for the formulation containing the lowest amount of poloxamer 188. The results suggested that higher concentrations of poloxamer 188 would reduce the hydrophobicity of the SLNs and decrease lymphatic uptake of carvedilol, which would result in lower oral bioavailability of the drug.<sup>127</sup>

## Conclusion

Advances in current approaches to lymphatic delivery of lipid-based nanoformulations have been reviewed. The lymphatic route provides new possibilities for delivery of cytotoxic agents and therapeutic molecules with higher first-pass metabolism and lower solubility. This method can serve as a bypass route, especially for anticancer and anti-human immunodeficiency virus drugs, both of which target diseases utilizing the lymphatic system. Drugs that are encapsulated in advanced lipid-based nanoformulations, such as NLCs, are better candidates for lymphatic drug delivery. With appropriate optimization and selection of an effective administration route, lipid-based nanoformulations should have great promise as lymphatic drug delivery systems.

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## Disclosure

The authors report no conflicts of interest in this work.

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