

# The Lymphatic System: A Sometimes-Forgotten Compartment in Pharmaceutical Sciences

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**ABSTRACT** -- The uniqueness of structure and physiology of the lymphatic system make it challenging to delineate all its contributions in the maintenance of our health. However, in the past two decades, the understanding of the importance of the function of this system has evolved and more appreciation has been drawn to the distinctive role it plays in health and disease. The lymphatic system has been linked to the pathophysiology of numerous ailments including cancer, various metabolic diseases, inflammatory conditions, and infections. Moreover, it has also been revealed that lymphatic targeted formulations can enhance the delivery of drugs through the lymphatic system to the bloodstream, bypassing the hepatic first-pass metabolism if taken orally, thus increasing the bioavailability, and improving the pharmacokinetic and toxicological profiles in general. Engineering lymphotropic preparations requires the understanding of many factors, the most important one being that of the physiological environment which they will encounter. Therefore, in this review, we detail the basic structure of the lymphatic system, then highlight the therapeutic and the pharmacokinetic benefits of drug delivery into the lymphatic system. The criteria for drugs and formulations used for lymphotropic delivery are also detailed with a contemporary overview of various studies undertaken in this field.

## OVERVIEW AND MAJOR MILESTONES

About 20-30 litres of plasma are propelled daily out the arterioles into the interstitial spaces of the body tissues. Of this volume, about 90% is reabsorbed back through the venules (1). The remaining fluid is drained back to the circulation via the lymphatic vessels. These vessels, in addition to other tissues and organs, form the lymphatic system (1-3).

The lymphatic system primarily maintains fluid homeostasis but also plays a pivotal role in transporting dietary fat and lipophilic molecules and entities from the intestine to the bloodstream. Moreover, it is involved in all immunological processes and numerous diseases and metabolic disorders which will be discussed later in this review (4-6).

It was Thomas Bartholin who first gave the term lymphatics to this system in 1652 (7). Nevertheless, the earliest recognition of the lymphatic system dates to the 4<sup>th</sup> century B.C.E. by Hippocrates and Aristotle (8). Throughout the following centuries, the importance of the lymphatic system with respect to health was largely overlooked. It was not until 1622 when

this system regained recognition and was described by the Italian physician Gasparo Aselli who found the intestinal lymphatic vessels, that he called “lacteals” while dissecting a dog’s abdomen (9). Aselli’s work was published in 1627 after his death, and that was one of the many landmark discoveries in the 17<sup>th</sup> century; the golden era for lymphatic system research (9, 10).

Again, key gaps in knowledge about various aspects of the lymphatic system remained understudied for a long time afterwards (10). Yet, three decades ago, the lymphatic system started gaining more scrutiny and interest. Advances in science have led to the salient understanding of the role of lymphatics and its link to numerous diseases. (10, 11). Major milestones in lymphatic system research spanning centuries are summarized in Table 1.

## STRUCTURAL ORGANIZATION OF THE LYMPHATIC SYSTEM

The fluid surrounding body’s cells is termed the interstitial fluid. When this fluid enters the lymphatic system, it is referred to as “lymph.” It does so through the blind-ended lymphatic

capillaries, which are sometimes termed, the initial lymphatics. From there, lymph drains into the collecting vessels, which passes through at least one, but usually several lymph nodes distributed throughout the body. Collecting vessels merge into grander trunks which empty into the ducts. Finally, the ducts return the lymph into the venous circulation, completing the circuit of fluid transport (1, 3, 4).

**Table 1.** Milestones in lymphatic system discovery and research throughout different eras

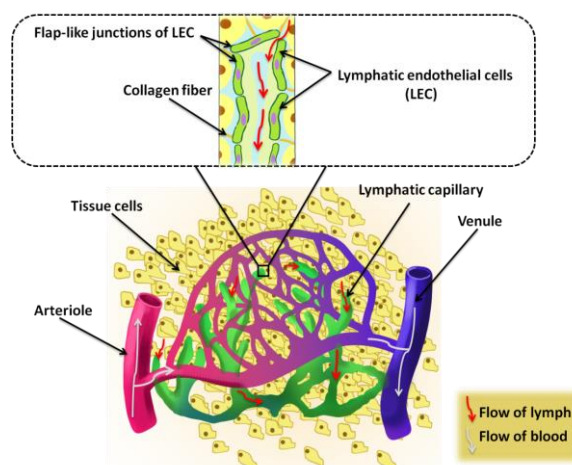
Year	Marks related to lymphatic system
460-377 B.C	Hippocrates recognized some lymph nodes in various body areas containing a “fluid absorbed from the tissues” (8)
300 B.C **	Aristotle’s detection of a lymphatic vessel. He described them as “fibres” between nerves and veins (8).
1622	Discovery of gut lymphatics by the Italian Physician Gaspare Aselli. He called them “venae albae aut lacteae” or (lacteals) (9).
1651	The French Physician Jean Pecquet described the thoracic duct and its valves. He also recognized that gut lymphatics empty into the cisterna chyle and not the liver as previous anatomists claimed (12).
1652-1653	Thomas Bartholin, a Danish anatomist, coined the term lymphatic for the first time which appeared in his book “vasa lymphatica.” He also confirmed the findings of Pecquet and illustrated that lymph from the intestine flows till it reaches the thoracic duct and that from the liver also do so separately (7).
1744	Description of the morphology and function of the lymphatic valves by the Dutch botanist and anatomist Frederik Ruysch (12).
1784-1787	Paolo Mascagni demonstrated the lymphatic network of the entire body (13).
1869	Arnold Heller noted the first description of lymph propulsion, observed in collecting lymphatic vessels in the guinea pig mesentery (14).
1962	3D graphic illustration of the liver lymphatics by Leonetto Comparini (10).
1992-present	Discovery of the growth factor/receptor system and related findings by Kari Alitalo, his team, and other international teams (10).
2015	Discovery of the brain lymphatic system by a team led by Drs. Antoine Louveau and Jonathan Kipnis from the University of Virginia School of Medicine (15)

## Lymphatic Vessels

**Lymphatic Capillaries.** Lymphatic capillaries, initial or terminal lymphatics are commonly interlaced with the capillaries in the connective tissues of various parts of the body except for bones and teeth (16, 17). These are blind-ended structures that are a one cell-thick layer of thin-

walled endothelial cells (18). These cells are 10-60 µm in diameter and possess a unique oak leaf shape (17, 19). They have a discontinuous or absent basement membrane acting as primary valves and overlapping button-like junctions (Figure 1) (20).

Anchoring fibres tie the initial lymphatics to the extracellular tissue matrices (21, 22). These elastic filaments protect the initial lymphatic from collapsing under high tissue pressure. They also sense the pressure in the intercellular space and signal the opening of the flap-like junctions for drainage of the lymph (19, 23).



**Figure 1.** Illustration showing the lymphatic capillaries interlaced with the blood capillaries network (Left). The lymphatic capillaries or the initial lymphatics (Right) are closed-ended vessels, composed of a single layer of epithelial cells having flap-like junctions in between that serves as valves allowing the interstitial fluid to be drained into the lymphatics. These capillaries are anchored through filaments to the surrounding tissue which help them withstand the high pressure without experiencing a collapse. From [https://commons.m.wikimedia.org/wiki/File:Illu\\_lymph\\_capillary.png#mw-jump-to-license](https://commons.m.wikimedia.org/wiki/File:Illu_lymph_capillary.png#mw-jump-to-license)

**Lymphatic Precollectors.** They are lymphatic vessels built of an endothelial layer of cells (19, 24). In regions close to the initial lymphatics, the endothelial cells of the precollectors still retain the oak leaf shape. The closer proximity to the collecting lymphatic vessels, the endothelial cells acquire a rhombic form like that of the veins (6, 25).

These vessels resemble the initial lymphatics in having no smooth muscle layer. Yet, they differ from them in having structured valves within, which are termed secondary valves. The valves here serve in preventing backflow into initial lymphatics (6). Having no smooth muscle layer, lymphatic precollectors rely on the inflow

and outflow pressures of individual segments in pushing the lymph forward (3).

**Collectors.** The next structural vessel organizations in the lymphatic system are the collecting lymphatics. The walls of which are composed of an endothelium layer encircled by a smooth muscle cell layer and a layer of collagen fibres; the adventitia (26).

These lymphatic vessels get thicker as they merge. A new feature of these lymphatic vessels is the microcirculation or the "vasa vasorum" that delivers oxygen and nutrients to larger collectors (27). Moreover, the secondary valves here play structural and functional roles. They prevent lymph retrograde movement and also divide the collecting vessels into chambers (3, 26). Each chamber forms a contractile unit called the lymphangion, meaning "lymph heart". Contraction of lymphangions together with the proper functioning of the valves ensures the smooth unidirectional flow of lymph even against gravity in a standing posture (28).

Usually, many collectors drain into a lymph node, and typically but not always one collecting vessel exits the node. The former is the pre-nodal or the afferent lymphatics and the latter is one of the post-nodal or the efferent lymphatic vessels (29).

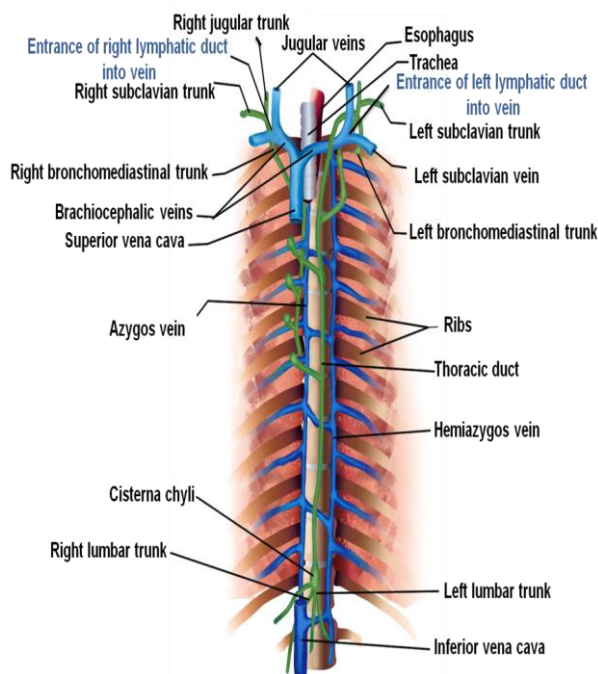
**Trunks.** Structurally, they are the larger counterparts of the lymphatic collectors (29). But functionally, there are some differences; in humans they funnel into two major ducts that return lymph back into the venous circulation. Two major trunks; the intestinal and the lower lumbar, drain into a sac-like structure called the cisterna chyli, located at the base of one of the ducts; the thoracic duct (3, 30). Other major trunks (jugular, subclavian and broncho mediastinal) drain directly into ducts (31) as shown in Figure 2.

**Ducts.** The last part of the lymphatic network is the ducts. Eventually, the larger vessels merge into the lymphatic trunks, that empty into the venous circulation via the right lymphatic duct and the thoracic ducts (2) as depicted in Figure 2.

The right lymphatic duct is formed from the merger of the right jugular, the right subclavian, and the right bronchomediastinal trunks (29). It receives lymph from the right sides of the head, thorax, and right upper limb and drains into the junction of the right subclavian and right internal jugular veins (32). Lymph from the remaining parts of the body get through the thoracic duct

into the junction of the left subclavian and left internal jugular veins (33). It is built of smooth muscle fibres and has a valvar system to prevent lymph backflow and blood reflux at the point where it meets the venous system (33, 34).

Summary of the order of lymph flow through the lymphatic network and the structure of each vessel is illustrated in Figure 3.



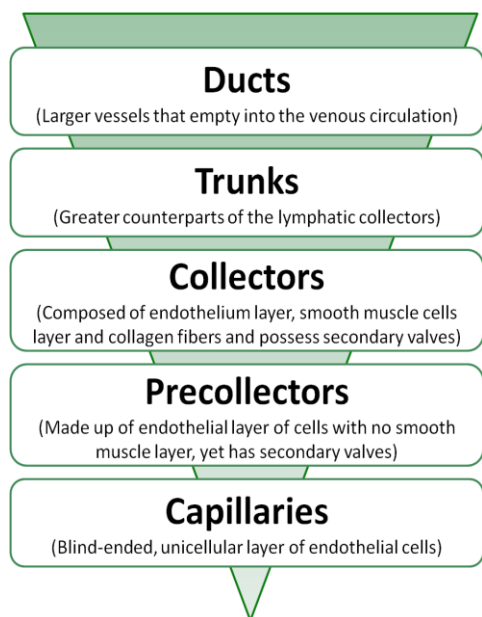
**Figure 2.** Major lymphatic trunks and ducts in the human body. The intestinal and the lower lumbar trunks empty into the cisterna chyli, before draining into the thoracic duct. Whereas the jugular, subclavian and broncho mediastinal trunks funnel directly into the duct located on their side. The right lymphatic duct drains lymph into the junction between the right jugular and subclavian veins, whereas the thoracic duct empty into the junction of the same veins on the left side. From (29) with permission.

### Lymphatic Tissues and Organs

The lymphatic tissues and organs are grouped as primary and secondary based on their functional roles (29). The primary lymphatic organs are the sites of lymphocyte formation and acquisition of immunocompetency and include the red bone marrow and the thymus (29, 35).

The secondary lymphatic organs are where immune responses occur. Lymphatic nodes, spleen and lymphatic follicles are members of this group (35). The first two (the lymphatic nodes and the spleen) are classified as organs because they possess an outer capsule of connective tissue, unlike the lymphatic nodules which lack it and as a result are considered tissues (29). There are numerous lymphatic nodules

found within the mucous membranes lining the respiratory, gastrointestinal, and urinary tracts, termed mucosa-associated lymphatic tissues (MALTs) (36). Clusters of these nodules can be found in the tonsils and in the ileum of the small intestine these are referred to as Peyer's patches (37).



**Figure 3.** Lymphatic network of vessels starting from capillaries and ending with ducts.

Out of these different lymphatic organs and tissues, lymph passes only through the lymph nodes which will be detailed next.

**Lymphatic Nodes.** Lymphatic nodes resemble immunosurveillance units, which functionally serve in filtering the lymph and mounting immune responses against detected antigens (6).

These nodes are bean-shaped lymphoid organs placed throughout the body, most prominently near the mammary glands and in the axillae and groin. Lymph nodes range between 1 to 25 mm in length and are divided structurally into two major parts, capsule, and parenchyma (Figure 4). The capsule is a dense fibrous tissue that runs towards the interior of the node forming partitions called the trabeculae. The parenchyma has two distinct sections: the cortex and the medulla (35, 38).

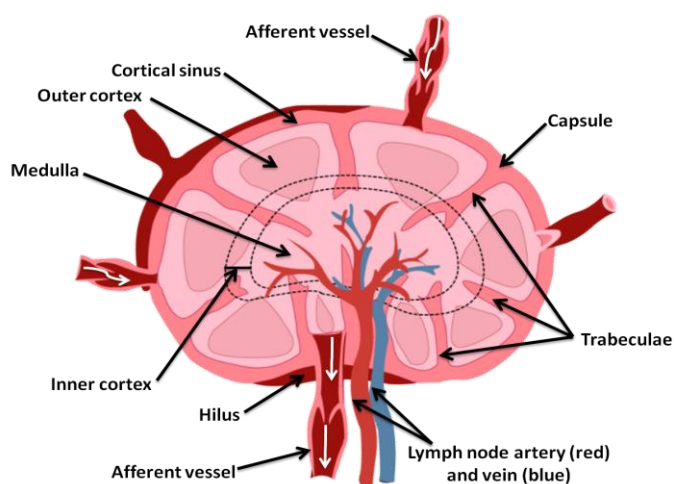
The outer cortex houses lymphatic follicles or nodules. There are two types of lymphatic nodules: primary and secondary nodules (39, 40). The former consists of B cells surrounded by a loose network of dendritic cells. Upon encountering an antigen, the macrophages or the dendritic cells stimulates the development of the

secondary nodules by the activation of the B cells which are bounded by cortical dendritic cells and macrophages form what is termed a germinal centre. Surrounding this centre there is a condensation of B cells, forming the outer part of the secondary nodule (40).

There are no lymphatic nodules in the inner cortex; instead, there are T cells and macrophages that migrate from other parts of the body. The macrophages cause the proliferation of T cells to combat antigens. The activated T cells do not reside in the lymph node but rather travel where there is antigenic activity (41).

Cells in the medulla are the antibody-secreting plasma cells that proliferate from the activated B cells in the outer cortex, in addition to the macrophages (29, 42).

Lymph drains into the nodes via the afferent vessels entering the nodes through its convex side. It follows a certain path crossing the sinuses within the node then exits through the efferent vessels emerging from the hilum (a depression on the concave part of the lymph node) (43). Cells might get into nodes with the lymph or through special blood vessels termed high endothelial venules (HEVs) (4).



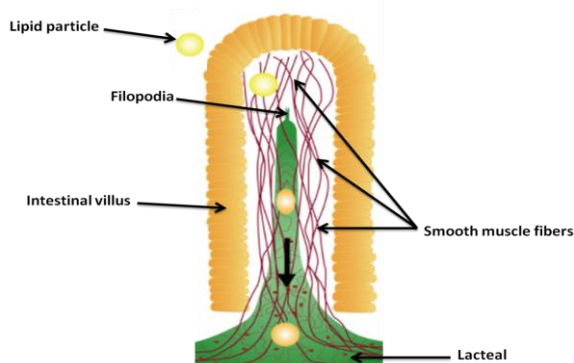
**Figure 4.** Structure of the lymph node. It is composed of the capsule and the parenchyma. The parenchyma encompasses the cortex and the medulla. The cortex is divided into outer and inner parts. The former contains the primary and the secondary follicles. The secondary follicles differ from the primary ones in having a germinal centre (activated B cells with dendritic cells and macrophages) surrounded by a condensation of B cells. Moving inwards there will be T cells and macrophages in the inner cortex, and antibody-secreting plasma cells with the macrophages in the medulla.

## Intestinal Lymphatic System

The intestines are a part of the gastrointestinal organ system and a region in the body that exhibits unique morphology and function of the lymphatics that is not encountered anywhere else. Here the lymphatic system besides drawing out excessive fluids and mounting immunological responses, which are the roles of the lymphatics throughout the body, also facilitates the absorption of dietary lipids through its special lymphatic capillaries; the lacteals (44, 45). In addition, there are several vitamins and food nutrients that use this system to access and enter the systemic circulation.

Structurally, the intestinal lymphatics start with the lacteals, found in the intestinal villi. The lacteals funnel into pre-collecting and collecting vessels located in the mesentery, which in turn drain into the cisterna chyli at the posterior end of the thoracic duct (45).

As illustrated in Figure 5, lacteals range between 60 and 70% of villi length and are encircled by a mesh of blood capillaries and smooth muscle fibres. A cytoplasmic extension or filopodia is usually attached to the lacteal tip demonstrating the state of regeneration that the lacteals can undergo (30).

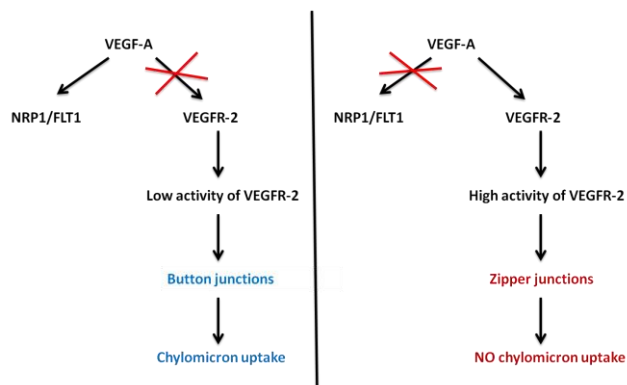


**Figure 5.** Structure of the intestinal lymphatic capillary (the lacteal). It constitutes nearly two thirds of the villi length and is surrounded by network of blood capillaries and smooth muscle fibres. It might have a cytoplasmic extension called filopodia, which indicates the state of active regeneration of the lacteals on which it appears. Lacteals play a vital role in up-taking absorbed lipids and draining them through the mesenteric lymph node into the thoracic duct before they enter into the systemic blood vasculature. Modified from (30) (Creative Common License).

The discontinuous button-like junctions between lymphatic endothelial cells of the lacteals indicates their functioning in lymph uptake. However, the transition to zipper-like cellular junction in the collecting lymphatic vessels

reflects less permeability and the better containment that these vessels must prevent the leakage while transporting lymph (18). Cellular junctions of lacteals were also linked functionally to the chylomicron's entry into the lacteal (46).

Chylomicrons are the form into which lipids and lipophilic components are assembled to be up taken by the lacteals (47). Following their absorption, dietary lipids are hydrolysed into fatty acids and monoglycerides, then re-esterified to triglycerides in the endoplasmic reticulum of the enterocyte's apical membrane (30, 45). The triglycerides, cholesterol, cholesteryl esters, phospholipids, and the apolipoprotein are packaged into chylomicrons and set out from the basolateral membrane of the enterocyte (30). In order to access into lacteals, chylomicrons do not diffuse passively as claimed earlier, but rather are taken up actively through a mechanism involving molecular signalling that is yet to be fully understood (48). However, the vascular endothelial growth factor-A (VEGF-A) has been shown to modulate the cell-cell junctions in lacteals and blood capillaries (49), regulating the lipid uptake process via signalling pathways starting with the binding to the vascular endothelial growth factor receptor-1 (VEGFR-1) and its co-receptor, the semaphorin receptor (NRP1) as depicted in Figure 6 (30).



**Figure 6.** Schematic model of cell-cell junctions in lymphatic endothelial cells (LECs) regulating chylomicrons uptake through lacteals. The availability of the vascular endothelial growth factor-A (VEGF-A) for binding the NRP1/Fms-related tyrosine kinase 1 (FLT1) on blood endothelial cells (ECs) results in having the button like junctions between the LECs that enables the chylomicrons uptake into the lacteals. When the opposite is encountered and the VEGF-A binds the vascular endothelial growth factor receptor-2 (VEGFR-2) on the LECs, that imparts the tight zipper junctions on the LECs and facilitates the transport rather than the uptake of the chylomicrons into the lacteals. Modified from (30).

Lacteals are not the only lymphatic feature present within the intestine. There is another key component there, which is the Peyer's patch. These lymphoid tissues are located within the mucosal lining in the intestine. They compose a gateway for lymphatic voyage similar to lacteals and are also an immune surveillance site that encounters various ingested immune elicitors like bacteria, viruses and other factors (4, 50).

### LYMPHATIC SYSTEM ADDING THERAPEUTIC BENEFITS

The overall lymphatic function is now thought to be associated with the pathophysiology of various diseases more than initially considered. Thus, lymphatics are an important target site for drugs and their delivery systems used in these conditions, especially lymph resident diseases such as cancer and some viral infections. Moreover, the lymphatic nodes play a central role in generating an immune response, thus they are considered a crucial target for vaccines (4, 42, 51, 52). For these indications, a new chapter in drug delivery has opened and various formulations have been developed while others are being investigated to target lymphatics through different routes of administration.

Next, the areas of intimacy between the lymphatic system and the pathophysiology of some disorders and diseases are summarized, with the various studies to exploit the lymph targeted delivery to add therapeutic benefits

#### Cancer

Being routes of trafficking through the body, lymphatic vessels are used by the malignant cells to spread. They are preferred over the blood vessels for this mission because of the lymphatic's broader vasculature, lower pressure gradient and higher permeability (53). Usually, the metastases occur in steps, the first of which is the colonization of the sentinel lymph node (4). This can occur through the pre-existing lymphatic vessels or newly formed ones resulting from the induction of tumour secreting growth factors (e.g VEGF-A, VEGF-C and VEGF-D) that stimulate the lymphangiogenesis (formation of new lymphatic vessels) to that node and beyond. Once in the first targeted node (a regional node), disseminating cancerous cells continue to do the same to promote more drainage of the growth factors to the node to aid in invading a distant node using the same strategy (4, 53-56). Other lymphatic markers have also been linked to cancer metastasis such as Prox-1 and Lyp-1 (56). Therefore, developing lymphotropic formulations

for chemotherapeutic agents and lymphatic biomarkers could enhance their therapeutic outcomes, in terms of target specificity, drug resistance and toxicity.

It has also been delineated that, Lyp-1 is a nano-peptide that binds a specific receptor (p32) which is highly expressed on tumor-related lymphatics, macrophages, and cancer cells (57). The lymphatic targeting of the Lyp-1 achieved via a self-micro emulsifying delivery system (SMEDDS) resulted in decreasing the tumor size in 4T1 Tumor-bearing mice. Concomitant administration of the same peptide with the cytotoxic drug doxorubicin (Dox HCl) exhibited a reduction of the cell viability from 74.3% to 49.6% after 48 hours of incubation in the MDA-MB-231 cell line. Thus, lymphotropic delivery of the Lyp-1 can be an effective way to combat tumors (58).

Another example is the cytotoxic anti-cancer agent doxorubicin that has achieved greater antitumor efficacy through a lymphotropic formulation. The subcutaneous administration of liposomal doxorubicin decreased the volume of auxiliary lymph nodes by 56.77% in comparison with the intravenous formulation that caused a 27.08% decrease in auxiliary lymph nodes size using rabbits inoculated with VX2 cells to develop breast cancer. Additionally, this resulted in an increase of the apoptotic cell count by 3.21 and 1.97-fold for the liposomal and the free drug preparations of doxorubicin, respectively. The inhibition of growth and the induction of apoptosis of tumour cells that the liposomal doxorubicin imparted was postulated to lead to higher lymphatic uptake of the developed subcutaneous formulation and the greater drug reaching the regional lymph nodes where metastasis occurs (59).

Moreover, the intravenous and oral administrations of a doxorubicin-quercetin conjugate (DoxQ) demonstrated 5 and 1.5-fold increase in the area under the curve (AUC) compared with the unconjugated standard drug treatment (Dox), respectively. The volume of distribution (V<sub>ss</sub>) imparted with the intravenous DoxQ was  $0.138 \pm 0.015$  where that of the doxorubicin was  $6.35 \pm 1.06$  L/kg in male Sprague-Dawley rats. The oral Dox Q delivery system resulted in double the amount of Dox in the mesenteric lymph fluid than the Dox. The transformed pharmacokinetics and improved oral bioavailability of the DoxQ were attributed to the lymphatic transport of the drug conjugate with the lymphotropic antioxidant flavonoid quercetin (60).

Paclitaxel is used for treating many cancer types, such as breast cancer, lung cancer, ovarian cancer among others (61). Reports on targeted paclitaxel nano-formulations supported its superior chemotherapeutic activity when administered through lymphatics. When incorporated in inhalable solid lipid nano-carriers (SLNs), paclitaxel resulted in tumour cells survival rate of (19.34%) compared with 87% cell viability when free paclitaxel was administered intravenously in a mice lung cancer model. Moreover, the inhalable SLN-paclitaxel showed no toxicity upon prolonged treatment and about 20 times less concentration to inhibit 50% of cell growth ( $IC_{50}$ ) than intravenously administered paclitaxel (62).

SLNs were also used for the cytotoxic agent etoposide. The study used mice with Dalton's lymphoma to compare the biodistribution of radiolabeled free and nanoparticle-based etoposide through three routes of administration, i.e., subcutaneous, intravenous, and intraperitoneal. Following 24 hour after administration, the subcutaneous route exhibited greater drug uptake by 8-fold and 59-fold than the intraperitoneal and the intravenous ones, respectively. Likewise, subcutaneous administration also showed a relatively low tissue distribution, suggesting lesser systematic side effects of the drug. Therefore, the subcutaneous injection was suggested to be a better route for administering chemotherapeutic drugs targeting lymphatic-related malignancies (63).

Intraduodenal administration of methotrexate solution and SLNs formulations came in favour for the nano-formulations regarding the chemotherapeutic effect as a 10-fold increase in lymphatic drug uptake was reported with drug-loaded SLNs as opposed to the free drug solution using dialysis membrane and rat models (64). Thus, the oral bioavailability of methotrexate can be improved via lipid-based formulations favouring lymphatic transport.

Zara *et al* also studied oral lymphotropic delivery for cancer drugs using SLNs taking idarubicin as a model drug. The Intraduodenally administered formulations of idarubicin showed a 21-fold increase in the area under the plasma concentration time curve compared with the drug solution. Again, the greater biodistribution in the lymphatic system appeared to serve in decreasing the idarubicin concentration in the heart and thus reducing its cardiotoxicity. The 30-fold increase in the elimination half-life of the idarubicin loaded SLNs suggested its potential use as a sustained release delivery system (65).

9-Nitrocamptothecin (9NC) is a potent antitumor agent that is used to treat hepatocellular carcinoma. The liposomal formulation of this drug (9NC-LP) has been shown to demonstrate a greater antiproliferative effect and fewer side effects in a nude mice xenograft model of HepG2 cell line in comparison with the free drug. The higher dose of the 9NC-LP (2.5 mg/kg/d) repressed cancer growth by nearly 87.02% and the lower dose scored 41.66% tumour growth inhibition after three weeks, without any drug-related death. Nevertheless, over half of the animals died on day 14 after administering 2.5 mg/kg/day doses of the free drug. The observed effects with the 9NC-LP systems were attributed to their lymphotropic delivery (66).

Hyaluronic acid (HA) is a natural polymer transported via the lymphatics and when coupled with the chemotherapeutic agent cisplatin (Pt), it made a successful local pulmonary delivery system with greater platinum concentration in the lung and draining lymph nodes as reported by Xie *et al* (67). A similar approach was considered with hyaluronan-cisplatin (HA-Pt) nanoconjugate to treat head and neck squamous cell carcinoma (HNSCC). In a developed orthotopic metastatic xenograft model of HNSCC, HA-Pt nanoconjugate achieved complete treatment success for 57% of the female mice whose group also showed significant hindrance of the HNSCC progression in contrast to the standard therapy group ( $p < 0.05$ ) (68).

All previous examples reinforce the importance of lymph-targeted delivery for cancer treatment. However, the lymphatic system is also related to other diseases, as discussed next.

### Inflammatory Conditions

Inflammation is a mechanism of protection against various pathogens and irritants (69). It is characterized by the expansion of both blood and lymphatic networks (angiogenesis and lymphangiogenesis, respectively). Whereas the proliferation of blood vessels exacerbates the inflammation, lymphatics were found to aid in containing the aggravation of this condition (70, 71). The reported mechanistic reasons underlying this was based on the formed lymphatic vasculature acting as clearance conduits, alleviating oedema, and decreasing the levels of pro-inflammatory mediators and immune cells (69). The molecular mechanisms involved in some inflammatory diseases like skin inflammation (72, 73), inflammatory bowel disease (IBD) (74) and rheumatoid arthritis (RA) (75) and others have been connected to lymphatic biology (69).

Developing delivery systems of lymphangiogenic factors would increase the potential of effective alleviation of inflammatory pathologies. Yet, limited studies are available on this targeted approach. A recent report showed that the antibody-mediated delivery of the vascular growth factor-C (VEGF-C) reduced skin inflammation in two mice models due to its accumulation in the affected tissues and stimulation of the expansion of the lymphatic vascular network (69).

Another study on the lymphatic related effects on inflammation involved the use of the tissue necrosis factor (TNF). It is a pro-inflammatory mediator that was linked to rheumatoid arthritis through its induction of neutrophils which when elevated and impairs the lymphatic pumping and aggravates the inflammation associated with rheumatoid arthritis. A study by Aldrich *et al* revealed that the intradermal administration of the anti-TNF drug, etanercept, improved lymphatic functioning and reduced the swelling in a rat model of collagen-induced arthritis (CIA) (76).

Intriguingly, many current treatments for inflammatory diseases, such as tocilizumab and infliximab affect lymphangiogenesis (77, 78). These proteins are administered through subcutaneous and intravenous routes and their relatively large size makes at least part of the administered dose to be taken up by the lymphatics (4). Thus, they would exhibit lymph related changes that account for their anti-inflammatory action. Yet, the door is still open for research in treatment options using the lymphatic system-inflammation overlap.

### Metabolic Diseases

Accumulating evidence supports the crosstalk between lymphatics and adipose tissue. The link between lacteal permeability and transport has been already established with adult obesity (46, 79). The disturbance in the signalling pathways modulating the cell-cell junctions of lacteals would result in leakage of fat-rich lymph and its accumulation, leading to diet-induced obesity (46). Insulin sensitivity has also been found to tie with the proper intestinal lymphatics functioning (80). Hyperinsulinemia and inflammation arising from obesity can also affect the lacteals integrity, progressing to deadly complications (30). Nevertheless, there is still an open area of research to dig in here and in other metabolic diseases which were connected to the lymphatic system such as hypertension (81), atherosclerosis (82, 83) and others (4).

In this disease group, there hasn't been translational development of drugs targeting lymphatics for the various indications that have been associated with the lymphatic system yet. Nonetheless, there are drugs that are being used to treat some of these metabolic diseases which have been found to have higher therapeutic efficacy when administrated through delivery systems that favour lymphatic transport. In the aforementioned studies, the reasons behind the better outcome were solely related to the pharmaceutical rather than the pharmacological reasons; with the lymphotropic formulations offering higher bioavailability and preventing the first-pass metabolism. A list of these drugs is in Table 3.

### Infections

As a system hosting immune surveillance centres and paving immune trafficking pathways throughout the body, the lymphatic system plays a vital role when antigenic invaders enter the body (3). Of special importance are the ones which take advantage of their accessibility to the lymphatics and utilize it to disseminate. Persistent HIV replication was connected to low lymphatic concentrations of the antivirals (84, 85). The list of other infections includes hepatitis (86), Ebola virus and recently the novel human coronavirus, SARS-CoV-2 was added to it (87). This data suggested lymphatics as a therapeutic target that would aid in eradicating certain challenging infections.

One example involves the link of the efficacy of the antivirals in downregulating the replication of the human immunodeficiency virus (HIV) in lymphatic tissue which was revealed to be associated with the lymph node concentration of antiretroviral drugs (88, 89). In support of this contention, the administration of subcutaneous nanoparticles in macaque monkeys indinavir extended the plasma residence times and increased the concentrations of indinavir in the lymph nodes of HIV-2 positive animals. This in turn caused a considerable reduction in the viral RNA load and an incremental increase in the CD4<sup>+</sup> T cell count (89).

### PHARMACOKINETIC BENEFITS OF THE LYMPHATIC SYSTEM

In addition to improving the therapeutic outcomes, lymphotropic delivery can add new possibilities for drugs of low solubility and those that are subjected to the hepatic first-pass effect. Such formulations can increase drug bioavailability, impart higher drug exposure, and lower toxicity by providing a by-pass route of the



hepatic circulation to enable greater systemic access (4, 51). Table 2 summarizes various studies that reported lymphotropic formulations used to improve the therapeutic effects for different indications by enhancing the pharmacokinetics profile of drugs.

## REQUIREMENTS FOR LYMPHOTROPIC FORMULATIONS

Formulations for lymphatic drug delivery, whether for targeting a lymph related disease condition or getting through the lymph to the general circulation- all have certain general requirements that affect their performance. These formulations include lymphophilic emulsions, microemulsions, self-emulsifying and self-micro emulsifying drug delivery systems. Additionally, numerous nanoformulations (e.g., polymeric nanoparticles, nanostructured lipid carriers, solid lipid nanoparticles and others) have also been considered (11, 53, 56).

Nano-sized formulations are found to be superior to actively target lymphatics, especially those which are lipid-based (53). Such formulations are better candidates for lymphatic drug delivery. However, the uptake of these formulations into the lymphatic conduits depends on some factors, such as the route of administration (4). The intravenous administration of cytotoxic agents is reported to result in limited tumour uptake of the drug due to a faster clearance by the phagocytic system (102). An example of such effect has been encountered with the etoposide (Section 4.1). In addition, the intradermal route may enhance the lymphatic uptake in comparison with the intramuscular and the subcutaneous routes because of the elevated interstitial pressure and faster flow of lymph within the skin than in other interstitial sites (103). Likewise, other features such as the particle size,

surface charge, hydrophobicity and types of lipids used also affect the lymphatic voyage (53, 56).

The molecular weight, partition coefficient, triglycerides solubility are the main criteria considered for drugs incorporated in lymphotropic formulations (53, 56). Other factors like surface area polarity and  $pK_a$  have also been reported to play a role in drug-chylomicron association and thus the intestinal lymphatic uptake (104).

Requirements for both drugs and formulations targeting lymphatics are summarized in Table 3.

## CONCLUSIONS

Over the last two decades, the functional importance of the lymphatic system in a wide range of diseases has become more evident. Thus, the lymphatic system itself is arising as a potential drug targeting avenue that could enhance the therapeutic outcome of such conditions. There are many studies that have focused on cancer and metastasis and the lymphatic system, however, translational studies on the lymphotropic formulations for therapeutic purposes is still in their infancy for many other disorders including inflammatory and metabolic ones in addition to various infections. Moreover, lymphotropic formulations have been linked to improved bioavailability and pharmacokinetic profiles especially for drug species liable to the first-pass metabolism. Optimized lymphatic targeted drug delivery requires a thorough understanding of the physiology of this system. Therefore, future studies would focus on understanding the detailed mechanisms of the entry and voyage of the lymph and mimicking the endogenous behaviour of lymph transported materials.

**CONFLICT OF INTEREST.** None.

**Table 2.** Lymphotropic formulations of drugs targeting various ailments.

Formulation	Drug	Main Indication	Remark	Reference
SMEDDS	Halofantrine	Antimalarial	Lymphatic uptake of the SMEDDS formulation reached 27.4%	(90)
	Valsartan	Antihypertensive	The AUC for the SMEDDS was 607 ng h/mL/hr in comparison to 445.36 and 1.36 h for market formulation	(91)

Table 2 continues .....

<b>Microemulsion/ SMEDDS</b>	Raloxifene	Osteoporosis Agent	<i>in vitro</i> intestinal permeability studies demonstrated that the microemulsion exhibited significantly higher permeation (90%) compared to the plain drug suspension (41.06%)	(92)
<b>Liposomes</b>	Cefotaxime	Antibiotic	Bioavailability of liposomal cefotaxime was approximately 2.7 times higher than that of the aqueous solution	(93)
	Tobramycin	Antibiotic	The AUC of tobramycin in SLN ~120-fold greater than that following IV administration of tobramycin solution	(94)
	Clozapine	Antipsychotic	Higher SLN bioavailability than that of the suspension	(95)
<b>SLNs</b>	Carvedilol	Antihypertensive	SLNs had higher uptake to the CaCo-2 cells than the drug solution owing to higher lymphatic transport	(96)
	Nimodipine	Prophylaxis of stroke and hypertension	SLNs conducted in male Albino Wistar rats showed 2.08-fold increase in relative bioavailability than that of drug solution, when administered orally	(97)
	Silymarin	For liver disorders	Greater bioavailability and lower hepatotoxicity noted with the SLNs of silymarin compared with commercial product	(98)
<b>Niosomes</b>	Rifampicin	Antibiotic	46.2% of the drug was taken into the lymphatic when the niosomes were administered intraperitoneal route in comparison with 13.1% for the drug solution through the same route	(99)
	Vinpocetine	For cerebrovascular disorders	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	(100)
<b>NLCs</b>	Testosterone	Hormone replacement	The lymphatically transported testosterone undecanoate accounted for between 91.5 and 99.7% of the systemically available ester	(101)

SMEDDS = Self micro emulsifying drug delivery system, SLNs = Solid lipid nano-particles, NLCs = Nano-structured lipid carriers, AUC= Area under the curve,  $C_{max}$  = Maximum plasma concentration.

**Table 3.** Criteria for drugs and formulations designed for lymphotropic delivery.

Formulation	Factor	Criteria Favoured Lymphatic Transport	Notes/Comments	Reference
	Type of lipid	<ul style="list-style-type: none"> <li>- Medium chain triglycerides (e.g., Caprylic triglycerides)</li> <li>- Long chain triglycerides (such as the ones in corn oil, olive oil, pea nur oil and soybean oil)</li> </ul>	The long chain triglycerides support the lymphatic uptake more than the medium chain ones.	(105)

Table 3 continues .....

<b>Carrier's Charge</b>	Negative (Zeta potential <-30 mV)	Negatively charged particles shows higher uptake into the lymphatics than neutral (zeta potential between +10 to -10 mV) and positive (zeta potential >+30 mV) counterparts. However, highly negatively carriers can extend the retention period of in the lymph nodes.	(106-108)
<b>Nano-particle's size</b>	10-100 nm	This is the optimal range for the lymphatic transport, however sizes greater than 100 nm can still provide lymphatic voyage, however at a slower rate.	(109)
<b>Hydrophobicity</b>	High	The higher the hydrophobicity of the formulation, the higher the lymphatic uptake.	(4)
<b>Emulsifier concentration</b>	< 1.5 % v/v	Concentrations higher than 1.5% v/v tend to decrease the lymphatic uptake.	(96)
<b>Molecular weight</b>	> 16,000 Daltons	Molecules of sizes < 10,000 Daltons are readily taken by blood capillaries rather than lymphatics.	(110)
<b>Drug</b>	<b>Triglyceride solubility</b> <b>Log P</b>	> 50 mg/mL > 5	(111) (111)

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