Drug Delivery Systems for Lymphatic Uptake

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The lymphatic system is considered to be the second circulatory system within the body, responsible for the maintenance of fluid homeostasis and immune protection. Among the aforementioned roles, it was proved that lymphatics are involved in dissemination of cancer and infections. This review offers a short description of the physiological features of lymphatic network, the lymphatic transport and the main drug delivery systems for lymphatic uptake.

Keywords: lymphatic system, circulatory system, cancer, infections, drug delivery systems

The last decades of research in the field of lymphatics were very fruitful in gathering insights into the morphology, anatomy, physiology and pathophysiology of the lymphatic systems components (organs, tissues and vessels). The published data offer a clear view regarding the biological roles of the lymphatic network, roles of great significance in maintaining the health status of the organism. The new techniques applied for lymphatic visualization, like magnetic resonance lymphangiography, also brought significant information about the localization of lymphatics and their role in cancer progression and metastasis via lymph nodes.

A great interest was bestowed on the role of lymphatics in development of solid tumors metastases and in immune surveillance against different antigens and diseases. Since the lymphatics are key players in cancer progression, there were developed drug delivery systems by the means of nanotechnology for lymphatic transport in order to target the lymph node metastasis and the secondary tumors located in different organs. The mechanisms of absorption and uptake at lymphatic level for different nano-formulations are not completely elucidated and understood, and further studies are required in order to complete the missing pieces.

The present review aims to present an overview regarding the biological roles of lymphatic system components, the characteristics of lymphatic transport and some data regarding the main types of drug delivery systems applied for lymphatic uptake.

A description of lymphatics and their biological roles

The lymphatic systemis a complex, one-direction, opennetwork that has a vessel tree appearance and consists of primary, secondary and tertiary lymphoid organs and tissues, and a tubular system of capillaries and vessels [1-4]. Another characteristic of this system is represented by the lack of a central pump unlike the circulatory system [4]. The bone marrow and the thymus gland, tissues responsible for the production of immune cells that provide the defense against different pathogens, form the primary lymphoid tissue. The secondary lymphoid system comprises the spleen, the lymph nodes, adenoids, tonsils, Peyer's patches and skin. At this level occurs an amplified and targeted immune response against a specific antigen by the resident lymphocyte immune cells.

The lymph nodes are considered to be the activation center of the immune response since its main activity consists in screening the lymph for foreign antigens. In contrast with the lymph nodes, the spleen does not have afferent lymphatic vessels, but is able to initiate immune responses against blood specific antigens by stimulating the lymphocytes and enhancing macrophage's phagocytic activity [1].The tertiary lymphoid tissues are located in the areas of chronic inflammation that lack lymphoid organs and their morphology is similar with the secondary lymphoid organs, whereas their functions are not fully elucidated [1]. Another component of the lymphatic system is considered to be the lymph, defined as the fluid that circulates throughout the lymphatic network and presents a composition similar to the interstitial fluid [5].The differences between the lymph and the interstitial fluid are represented by a higher concentration of proteins and the presence of large bacterial cells within lymph composition (fig. 1) [5].

Regarding the vascular lymphatic system structure, there were defined the following types of vessels: lymphatic capillaries, initial lymphatics and terminal or peripheral (collecting) lymphatics [2, 3].The lymphatic capillaries are distributed in most tissues of the organism and are lacking in the tissues that are not directly supplied with blood. They are characterized by a high permeabilityfor proteins, viruses and other big molecules with a reduced rate of diffusion, as a result of the poor cell-cell binding and the discontinuous basement membrane, their main role consisting in providing the fluid exchange [1, 2, 5]. The capillaries cohere and form pre-collecting lymphatic vessels with *zipper-like* adherent junctions between the lymphatic epithelial cells [1, 4].

The initial lymphatics vessels are considered to be the site where the lymph is formed. These vessels present fine

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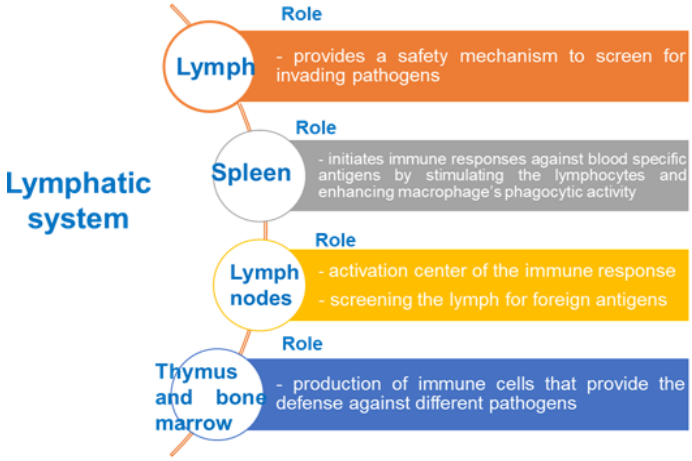

Fig. 1. Lymphatic systems organs and tissues and their biological roles

walls that combine a single layer of endothelial cells with an incomplete basement membrane, and are organized in interconnected meshworks with tissue specific characteristics [2, 3]. An interesting feature of the endothelial cells from this level is the presence of *button-like* specialized intercellular junctions that exhibit VE-ecadherin and PECAM-1 adhesion proteins responsible for the development of coated leaflets of cell membrane between adjoining cells. Moreover, these structures were described to behave as one-way microscopic valves, also known as *primary valves* and they control the entrance of the fluids, macromolecules, and lymphocytes into the lymphatic lumen [2, 6, 7]. The water transport across the initial lymphatics cells is assured by the water channel aquaporin-1 that is highly expressed in the endothelial cells of initial lymphatics [2]. The initial lymphatics merge into larger, collecting lymphatics.

The collecting lymphatics vessels present a different structure as compared to the initial ones, as follows: endothelial cells, smooth muscle cells organized in layers, a tunica formed of fibroblasts, major histocompatibility complex class II (MHC - II) molecules and antigen presenting cells, and nerves [2]. It is notable to mention that these lymphatic vessels are constituted of multiple regions, termed lymphangions that intercommunicate by the means of *secondary valves* which have a semi-lunar shape, are unidirectional and prevent the backflow of the lymph [1-3]. The lymph collected by the collecting vessels is delivered to the lymph nodes, that are widespread throughout the body, and from there the postnodal collecting lymphatics cohere into two large lymphatic ducts: the right one (covers the drainage of the lymph from the right arm, the right side of the head and the right thoracic cavity and is unloaded into the right subclavian vein) and the thoracic duct (collects the lymph from all the remained parts of the body and is emptied into the left subclavian vein) that deliver the fluid back into the venous circulation [2, 5]. The collecting vessels function as peristaltic pumps for the transport of the lymphatic fluid toward the right and thoracic ducts with the help of the precisely coordinated contractions of the lymphangions' smooth muscle cells while the secondary valves prevent the reflux of the fluid [8].

The lymphatic network exerts multiple relevant roles within the body on the basis of itscomplex structure and its wide distribution within the body. Among the many functions exerted by the lymphatic system, one could mention the following: (i) to conserve the tissue – fluid homeostasis by draining the interstitial fluid from peripheral tissues to the venous circulation; (ii) plays a considerable rolein immune system's recognition and response to different antigens and diseases; (iii) as transport route for immune cells and soluble antigens from periphery to lymph nodes; (iv) in formation, gathering and transportof the lymph and (v) as an active player in cancer progression and infection dissemination [1-4, 9, 10].Recent advances in the comprehension of the lymphatic system roles in health and disease, led to the conclusion that the lymphatics are more likely active participants in the development and progression of these processes [8]. Another role of the lymphatic system consists in reabsorbing a significant percent (more than 50%) of the proteins that extravasate from the bloodstream [11].A defective function of the lymphatic vessels encompasses to several pathologies, such as: a local compromised immune response, metabolic disorders and lymphedema [3].

Liymphatic transport

The lymphatic transport starts in lymphatic capillaries that are spread in most tissues and organs,where the lymph is drained; the capillaries merge and form the initial lymphatics and the lymphatic fluid passes from here into the collecting lymphatics that present multiple valves which act as pumps [3, 12].The pumping mechanism exerted by the lymphatic system resembles the venous flow mechanism. The lymph flow across the lymphatic vascular system can be described, as follows: when the lymphatic vessels are filled with lymph, they extend, the extension leading to the activation of the smooth muscle cells that wrap the lymphatic vessels which automatically contract and propel the lymph into the lymphangions delimited by valves that prevent the backflow of the fluid [3, 5, 13].The movement of the lymph can be also increased by the contractions of any tissue that sends compressive load to the lymphatic system.The lymph is transported to the lymph nodes by the afferent lymphatic vessels, and passes through the lymph nodes sinuses where comes into contact with the immune cells and is screened for antigens [4, 5].

In order to verify if variations in lymphatic system dimensions could influence the lymphatic pumping efficiency, there were performed mathematical studies which showed that the optimum length of lymphangion is between 13 and 14.5 time its diameter [14, 16].

The total flow rate of the lymphatic fluid is about 125 mL/h, the volume of lymph that is formed daily and restored to the bloodstream reaches 3L.The process of lymph formation consists in the passage of water and nutrients

from the capillaries into the extracellular space and is considered to be the filtrate of the interstitial fluid. The lymph is described as a watery clear fluid, with a similar composition as the interstitial fluid when it enters into the lymphatic capillaries, its components changing with its movement along the lymphatic system where takes contact with different cells, proteins and molecules specific toevery transited tissue [5, 12]. The lymph formation process can be influenced by several factors: hydrostatic pressure of the capillary and of the interstitial space, and by the osmotic pressure within the capillary and the interstitial space. The lymph flow through the lymphatic network is in a direct proportional relation with the interstitial hydrostatic pressure [5].

The transport of lymph through the collecting lymphatics is governed byboth extrinsic and intrinsic forces. The intrinsic forces are represented by the phasiccontractile activity of lymphangions(the phases consist of brief periodsof rapid forward movement of fluid and an interphase of either no flowor very slow flow in the forward or reverse direction), whereas the extrinsic forces represented by high rates of lymph formation are responsible for periods of forward flow in the absence of lymphangion pumping [2, 17-19].The lymphatic contractions needed for the lymph flow are driven by Ca^{2+}fluxes from extracellular and intracellular depots and by mechanical forces, respectively [3].

The lymphatic transport of the drugs can occur at different levels dependent on the administration pathway: intestinal or cutaneous.The intestinal lymphatic system is preferred for the transport of large molecular weight drugs that are unable to pass through the blood capillaries, fat-soluble vitamins, food-derived lipids and water-insoluble peptide-like molecules orally administered, into the portal circulation. This type of transport presents several advantages, like: (i) avoidance of the hepatic first-pass metabolism what increases the oral bioavailability of the drug and (ii) an enhanced effectiveness for immunomodulatory and chemotherapeutic drugs, the lymphatic system being the main route for metastasis of solid tumors [12,20].

Highly lipophilic drugs administered orally present an increased entry into the lymphatic circulation at mesenteric level as compared to the bloodstream. This type of drugs form complexes with the chylomicrons, the proteins secreted by the enterocytes, that enter the lymphatic circulation avoiding the effect of first-pass metabolism at liver level and increasing the concentration of the drug into the lymph nodes and ducts [12, 20].

The intestinal lymphatic drug delivery is influenced by the presence of the food, the post-prandial administration augments the lymphatic transport of the molecules at intestinal level [12].

Drug delivery systems used for lymphatic uptake

In the view of the considerable progress that was achieved in the field of lymphatic system, in terms of anatomy, physiology and pathophysiology of lymphatic tissues, organs and vessels, and their biological roles, a great interest was assigned to the employment of this route for drug delivery systems, especially for anticancer and immunomodulatory drugs. One of the main reasons in performing this kind of assays were: the active role played by the lymphatic vessels in the dissemination of cancer cells to the development of metastasis and its function in

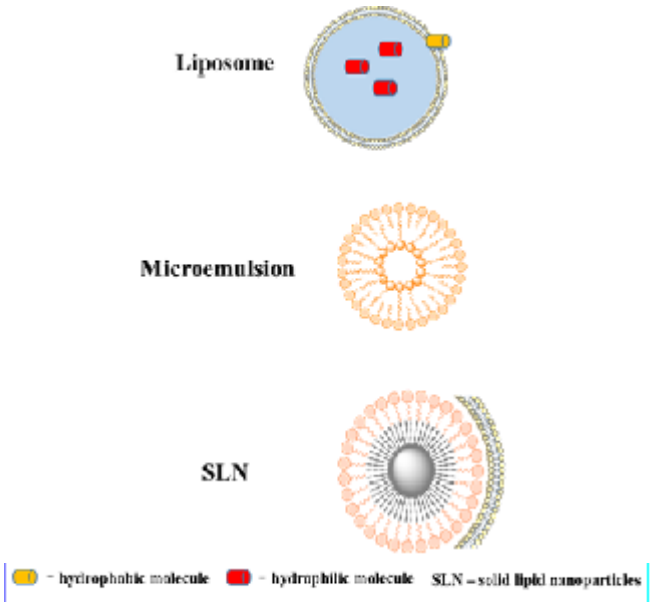

Fig. 2. The main nanocarrier systems for lymphatic drug delivery

production and activation of immune cells, and immune surveillance.

In the past, the diseases related to lymphatics were treated indirectly via drug delivery to the systemic circulation followed by drainage from the interstitial fluid into the lymphatic capillaries [21]. In order to prove the potential of lymphatic network as an efficient route for the delivery of different biologically active molecules there were proposed different targeted drug delivery systems, like: emulsions, classical and engineered liposomes, dendrimers, exosomes, solid-lipid nanoparticles, nanocapsules, etc. (fig. 2),that offer several advantages, including: an increased drug concentration at the site of disease, a longer systemic exposure to the drug by the lymphatic transport to the bloodstream; access for the large molecular weight lipophilic molecules (proteins, lipoproteins, synthetic macromolecules and nanoparticulate drug delivery vectors) via the endothelial lymphatic cells from periphery and gastrointestinal submucosa [9, 21-23].

For the development of successful drug delivery systems for lymphatic uptake, there must be complied several criteria: the drug delivery platforms design should avoid the reticuloendothelial uptake, must access preferential the lymph and the active molecule should be unloaded at the target site [21].

The parameters that must be adjusted during the design of drug delivery systems for lymphatic uptake and transport are: the size and the molecular weight of the molecule, the electrical charge and the hydrophobic/hydrophilic character. It was stated that an increased size of the molecules enhances the lymphatic uptake, whereas an increased hydrophobicity is associated with retention in lymph nodes. Moreover, by enhancing the hydrophilicity of the molecules it will improve their lymphatic drainage [21].

Several studies proved that dendrimers are an attractive platform for the development of lymph targeting vectors. The dendrimers are polymers obtained by addition of multiple layers that possess a central active core. Its structure presents similarities with the proteins, from here the name of *artificial proteins*. Dendrimers offer multiple assets as drug delivery systems due to the precise control of their molecular structure, modification of size, many

reactive sites, polydispersity and central void space [9, 21]. These nanomaterials were used as contrast agents in magnetic resonance lymphangiography (PAMAM – Gd containing dendrimers) [9, 24]. There were also obtained polylysine coated and PEGylated dendrimers that were well absorbed into the lymphatic system after subcutaneous administration [9, 21, 25].

Other types of nanomaterials that were verified as drug delivery platforms for lymphatic uptake are the engineered liposomes and solid lipid nanoparticles [26]. The liposomal formulations effectiveness as carrier platforms for lymphatic uptake was tested after subcutaneous, intestinal and pulmonary administration. The subcutaneous injected liposomes were used as vehicles for anticancer agents, and it was shown that exhibited a more potent pharmacological effect as compared to the ones administered via parenteral route. One of the disadvantages of liposomal formulations is the low retention time in the lymph nodes and it was designed a novel liposome coated with avidin-activated biotin what led to a prolonged lymphatic retention [12, 26]. The liposomal formulations for the lymphatic uptake via the intestinal route were applied for hydrophilic drugs that had a poor lymphatic uptake and a reduced intestinal bioavailability, the result being an augmented intestinal bioavailability and an increased lymphatic transport. The pulmonary route was chosen for the delivery of chemotherapy in order to destroy the metastases from this level, but also for the administration of nanoradioliposomes for the visualization of the lymphatic network [26, 27].

Solid lipid nanoparticles (SLN) are a well-known class of nanocarriers, obtained from physiological lipids (fatty acids, steroids, waxes, etc), characterized by a high stability and low toxicity. Administration of SLNs intravenously indicated a reduced uptake by the lymphatics, these data leading to the choice of alternative routes for lymphatic uptake: the duodenal route, the subcutaneous and pulmonary routes [12, 26]. The SLNs distribution along the lymphatics is dependent on the size, surface charge and hydrophobicity of the particles. For a good passage from the subcutaneous site of injection into the lymphatic vessels the SLN must have a size between the range 10-100 nm, a bigger size determining to the retention of the particles at injection site and a size < 10 nm will be absorbed into the blood circulation. It was shown that the negative charged and hydrophobic SLN are easily drained into the lymphatic vessels [26].

Vicente *et al.* showed in a study using rabbits that polysaccharide-based immunostimulating nanocapsules formed a depot at the injection site and exhibited an immunostimulatory effect. Moreover, it was observed that the injected nanocapsules were drained slowly into the lymphatic system and the retention time into the lymph nodes was prolonged [28].

Recent studies demonstrated that the lymphatic uptake is a potential route not only for anticancer agents and immunostimulating platforms, but also for other therapeutic molecules, like antivirals. The group of Makwana proved that Efavirenz, an antiviral drug, formulated as SLN for lymphatic drug delivery, was detected in spleen, a major lymphatic organ and was absorbed in lymph via the chylomicron uptake mechanism [29].

Engineered liposomes (LyP-1-conjugated PEGylated liposomes, intralymphatic-targeted hyaluronic acid-modified nanoliposome) [30, 3], long chain nanolipid carriers for hepatic delivery through lymphatic pathway [32], polyaminoacid nanocapsules [33], microemulsions containing long-chain oil ethyl oleate [34], are other types of systems applied for the improvement of lymphatic uptake by increasing the stability and bioavailability of the active molecules.

Conclusions

The developments regarding the lymphatic system comprehension in terms of biological roles, transport and lymphatic-targeted drug delivery systems have evolved greatly in the last years, but there are still some gaps to fill concerning the mechanisms involved in these processes, and also the aspects regarding the safety profile of the novel drug delivery platforms after *in vitro* and *in vivo* administration need to be intensively studied.

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