REVIEW ARTICLE

Polyethylene glycol block polymeric micelle: A promising delivery vehicle for lymphatic targeting

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ABSTRACT

The lymphatic system (LS) plays an important role in the human immune system. The tumor cells and some viruses like human immunodeficiency virus spread through the LS to all body parts. Tumor cells can even form secondary tumors on organs other than the primary site of origin. Clinically, treatment of metastatic lymphatic tumors presents a great challenge owing to limitations of surgical resection and the low effectiveness of chemotherapy and radiotherapy. Hence, lymphatic targeting could serve as a better alternative treatment, ensuring improved bioavailability by avoiding hepatic first-pass metabolism and increased absorption of lipophilic drugs with reduced systemic side effect. Further, leaky vasculature of LS provides opportunity of passive targeting. Importantly, various nanoformulations such as nanoparticles, polymeric micelles (PM) with a size smaller than 100 nm are efficiently accumulated and transported through LS, owing to enhanced permeation, and retention effect. Recently, various amphiphilic polymers have been synthesized to prepare PM for lymphatic targeting. Acknowledging the wide range utility of lymphatic targeting, this review attempts to provide an insight into the recent advances in development of polyethylene glycol block polymer micelles as lymphatic targeted carriers.

Keywords: Cancer, enhanced permeation, retention, immunomodulator, lymphatic drug delivery, metastases

Introduction

Over the past few decades, the systemic circulatory system (SCS) has predominantly been explored and used for delivering the drug or formulation/s containing drug/s for the treatment of various diseases. Besides the SCS, there is another circulatory system in the vertebrates called lymphatic system (LS) which circulates a fluid known as lymph. The major functions of the LS include maintenance of the body's water homeostasis and the absorption of plasma proteins and particulate cellular matter from the interstitial fluid and return of these substances to the blood.[1,2] The lymph nodes (LN), part of LS, serve as site of induction of cellular and humoral immunity.[3] Recently, the scientific research arena is gaining more interest toward LS as a target for drug delivery, evolving the lymphatic targeted drug delivery systems (LTDDS). However, LTDDS offers more challenging goal due to distinctive anatomical features of LS posing more difficulties in achieving drug access to the intended site in LS.[2] Lymphatic drug targeting refers to targeting of drugs and therapeutic agents into the LS for the drug action in the LS itself or their transportation in the lymph to specific tissues of interest.

Drug delivery to LS is getting more importance as it provides several unique benefits related to its anatomy and physiology. LTDDS greatly enhances the efficiency of drug especially in case of hydrophobic anticancer drugs, reducing their systemic toxicity and non-specific organ toxicity.[4] Moreover, peroral delivery of macromolecules such as peptides and proteins can be efficiently achieved by targeting them or their carriers to the lymphatic regions in gastrointestinal like Payer's patches where they can be absorbed in spite of their large molecular

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weight.[5] Payer's patches, the most important structural units of gut-associated lymphoid tissue, are an important site of absorption of lipophilic drugs or lipiodol drug carriers after oral administration. The lymphatic absorption of a drug, after intestinal administration, provides an advantage over the portal blood route for the possible avoidance of liver pre-systemic metabolism. Thus, formulation of the drug as lipoidal carrier can achieve lymphatic targeting and enhances the bioavailability of drug.[6] The peculiar morphological attribute of lymphatic vasculature is the arrangement of a single layer of non-fenestrated lymphatic endothelial cells (LECs) that lines lymphatic capillaries. In contrary to blood vessels, the LECs have poorly developed basement membrane due to lack of adherens junctions and tight junctions. Due to this, LECs have gaps (30-120 nm) making linings of capillaries more porous that serves as an entry portal for macromolecules, drug carriers, pathogens, and interstitial fluid. Carriers <100 nm in diameter, can extravasate into the interstitial space get phagocytozed by macrophages, and are finally absorbed into LN.[7] Hence, this leaky vasculature of lymphatic capillaries facilitates the entry of macromolecules or drug carriers such as liposomes and nanoparticles.[8] Due to such fundamental functions or characteristics, many attempts have been made to develop LTDDS.

The LS plays an active role in pathogenesis of some diseases such as cancer, metastitial tuberculosis, and filariasis.[9] This route is used as disseminating pathways by tumor cells, bacteria, and viruses throughout the body. Cancer cells employ LNs as a house and migrate to the distant body organs forming secondary tumors. This migration of cancer cells from the primary site of origin to other tissues or organs of the body and form secondary tumor is known as metastasis, the unique feature of cancer cells.[5,10] The reports of the presence of human immunodeficiency virus (HIV) at all stages of the acquired immunodeficiency syndrome (AIDS) and HIV replication in macrophages of lymphoid tissue confirm an important role of LNs in the development of AIDS.[11]

For treatment of diseases with lymphatic involvement, it is desirable to develop approaches delivering diagnostic, immunomodulatory, and chemotherapeutic agents to LS or LNs.[12-17] Recently, the research arena has been putting more efforts for the lymphatic targeting of anticancer agents. Besides chemotherapeutic agents, other objectives include the transport of anti-inflammatory agents to the site of inflammation and also the transport of certain macromolecules such as recombinant human interferon alpha-2a, and small interfering RNA (Si RNA).[18-20] Various routes of administration such as oral, intramuscular, intradermal, intraperitoneal, subcutaneous, and pulmonary have extensively been explored to achieve lymphatic targeting.[5,21]

Distinct types of drug delivery systems have been designed and investigated for their potential of preferential delivery to the LS. Lipophilic drugs are thought to be mainly transported through LS and reported that coadministration of the drug with lipoidal carriers could intensify their transport through LS.[22] Hence, the targeting of drugs to LS achieved by employing lipoidal carries such as emulsions, liposomes, self-emulsifying drug delivery systems, self-micro emulsifying drug delivery systems, solid lipid nanoparticles, and nanostructured lipid carriers.[5] On the other hand, polymeric nanocarriers such as polymeric micelle (PM), microspheres, and nanoparticles have also been extensively explored to target LS.[2,23] This review discusses recent advances in development of PM to facilitate the lymphatic targeting and effective management of diseases which spread through LS.

The leaky vasculature of LS, as shown in Figure 1, forms the basis of passive targeting of drug carriers with a mean diameter of 10-100 nm due to enhanced permeation and retention (EPR) effect. On the other hand, active targeting employs various ligands attached to the corona of PMs. These ligands are designed to have a specific binding affinity toward receptors which exclusively expressed by tumor cells on their surface. For example, He *et al.* achieved active lymphatic targeting by designing calcium carbonate nanoparticles containing Si-RNAs which specifically bind to some vascular endothelial growth factor-c, expressed on the surface of LECs.[24]

PM for lymphatic targeted therapy

Since the 1990s, PMs have been developed and extensively investigated as drug delivery carriers. On contact with the aqueous environment, amphiphilic polymers spontaneously form PMs, reported to be thermodynamically stable systems with core–shell structures. Hydrophobic fragments of polymer form inner core providing a seat for hydrophobic drugs, e.g., anticancer and water insoluble drugs, thereby improving their aqueous solubility. The hydrophilic shell shields entrapped drugs from outside adsorption or degradation and avoid phagocytosis by RES, prolonging drug circulation time *in vivo*. Polyethylene glycol (PEG) has been reported to be used as a hydrophilic polymer for surface modification of nanocarriers to extend circulation time. Therefore, the attempts have been made to design and investigate the polymers containing PEG blocked with hydrophobic polymers such as polystyrene (PS), and phosphatidylethanolamine (PE) which further proven to be better for PMs for lymphatic targeting. Likewise, their smaller size (10-100 nm) of PMs, resembling that of natural viruses, aids not only in extravasation and improving accumulation in solid tumors after systemic injection but also in overcoming physiological barriers like lymphatic transport to LNs after intradermal injection. Furthermore, PMs prove their potential as

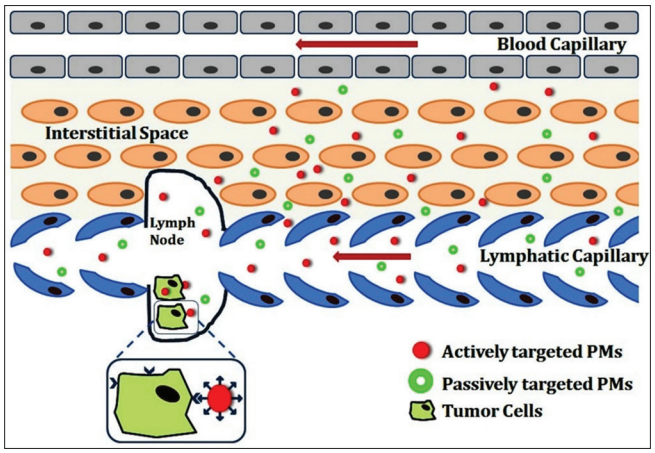

Figure 1: Leaky vasculature of lymphatic system and lymphatic targeted mechanism of polymeric micelles

passive lymphatic targeting owing to their optimal size. However, their surface can be ornamented with specific ligands for active targeting. This article provides a review of PEG containing PMs as LTDDS.

The LS plays active role in immune responses of the body. Therefore, Dane *et al.* formulated PEG-bl-poly(propylene sulfide) (PEG-bl-PS) micelle loaded with immunomodulatory drugs (rapamycin and tacrolimus) and anti-inflammatory drug (mometasone) intended to target LN resident T cells and dendritic cells (DC), which participate in survival or rejection of graft transplants. The kinetic study of micelle drainage to the LNs carried out by flow cytometry after intradermal injection of fluorescent label tagged micelle in mouse tail, and surprisingly the results showed a preferential association of micelle with LN resident T cells and DCs. Further, the skin allograft transplantation study in MHC-mismatched mice was performed to confirm immunomodulation by rapamycin micelle and the findings revealed that the graft survival time prolonged by 1.5 fold which was further prolonged to 2 folds by a combination of rapamycin and tacrolimus micelle as compared to that of empty micelle. On the other hand, DC activation study, after challenging with TLR9 Ligand, demonstrated successful mometasone delivery to DCs and prevented their activation from eliciting an inflammatory response against challenge. Hence, they concluded that the drug loaded PM approach is a valuable tool for lymphatic targeting to regulate immune functions of the body.[13]

On the same basis, Eby *et al.* postulated the activation of humoral and cellular responses following immunization with antigen coupled PM. In addition to above studies, this research group incorporated pyridyl disulfide, coupled to Ovalbumin, into the PEG shell of PEG-*bl*-PS to form Ovalbumin-conjugated PMs (Ova-PMs) for passive targeting to antigen presenting cells (APCs) in LS. Results of biodistribution studies of fluorescent Ova-PMs revealed their preferential association with APCs and accumulation in draining LNs, owing to their small size due to EPR effect. As presentation of antigen to APCs is enhanced, the enhanced productions of antibodies were intended in blood. Through immunization studies, they reported that, as compared to soluble antigen, immunization with Ova-PMs significantly enhanced the production of high-affinity antibodies by B cells and 70% more production of IFN-γ by T cells. These findings suggested the better potential of PEG-PMs as a lymphatic targeted delivery vehicle for antigen and reduction in dose of antigen for immunization.[14]

On the other hand, PEG-PMs can be employed for the treatment of cancer spreading especially through LS. For this purpose, Xiao *et al.* formulated doxorubicin-containing PM using series of PEG-oligocholic acid (CA) based telodendrimers. From the series of PEG-CA, the PMs prepared from PEG^{5k}-CA_4 (5k refers to molecular weight of PEG in Dalton, and 4 refers to number of CA subunits) exhibited higher drug loading (14.8%), superior stability, more prolonged release (up to 7 days) and have spherical shape with diameter of 10-20 nm. Cellular uptake and intracellular distribution of PMs was evaluated by confocal fluorescence microscopy and flow cytometry. The formulated PMs showed faster drug release in endocytic compartments than *in vitro* release and higher cellular uptake. Furthermore, the results of antitumor activities of formulated PMs in subcutaneous Raji lymphoma mouse model revealed their higher efficacy for the treatment of lymphoma as compared to free doxorubicin and Doxil®. Thus, doxorubicin-containing PEG-CA micelle could effectively be used for targeting the cancers of LS.[15]

Qin *et al.* made a similar attempt to evaluate the delivery of vinorelbine PEG-PE micelle (Nanovin) to reduce LN metastasis. Using real-time *in vivo* two-photon microscopy, they showed that nanovin remained intact and effectively extravasate from systemic circulation to interstitium and eventually transported to LN through LS. After i.v. injection of nanovin and accumulation of nanovin was found to be 4.7 times than that achieved after free drug administration after 24 h. Further, 4T1 spontaneous metastasis model was used to study the extension of metastasis from breast to nearby LN and organs like lungs. Surprisingly, it is observed that nanovin significantly reduce metastasis to nearby LN and increase the number of survived mice treated with nanovin. Therefore, their study suggested the potential of PEG-PE micelle as promising drug delivery not only to treat cancer at the primary site of tumor but also reduce their metastasis.[16]

In contrast to passive targeting, active targeting was thought to achieve higher specificity to specific receptors expressed exclusively on tumor cells. Based on this fact Wang *et al.* synthesized a block polymer of PEG-poly (3-caprolactone) (PEG-PCL) and prepared PMs for delivering artemisinin (A-PMs). The surface of PMs was decorated by conjugation with LyP-1, a cyclic peptide, which has the specific binding capacity to p32/gC1qR receptors on certain cancer cells like MDA-MB-435S breast cancer cell lines. Various studies confirmed that the LyP-1 modification not only significantly improve the uptake of A-PMs by both LEC and metastatic tumor cells but also enhanced cell apoptosis due to more blockade of cell proliferation cycle in S phase when results compared with that of A-PMs without LyP-1. Furthermore, *in vivo* studies indicated the higher specificity and anti-tumor efficacy to highly metastatic breast cancer and its related lymphatics of LyP-1 modified A-PMs than A-PMs. Collectively, the results demonstrated PEG-PCL micelles could be employed as a delivery vehicle for active targeting to LS.[17]

Conclusion

Advances in current approaches to employ PMs of PEG block polymers with various hydrophobic polymers as a delivery vehicle for lymphatic targeting have been reviewed. The lymphatic route provides new opportunities for delivery of chemotherapeutic agents and immunomodulators to achieve higher bioavailability circumventing their pre-systemic metabolism and low solubility. PM of various PEG block polymers was successfully used for effective treatment of diseases related to the LS. In conclusion, we believe that with appropriate optimization of size and corona modification, micelles of PEG block polymers would be a great promise as lymphatic drug delivery systems.

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