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Targeting to lymph nodes by subcutaneous administration of liposomes

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Abstract

An interesting route of administration for liposomes is the subcutaneous (s.c.) route of administration. Liposomes administered in this way can improve drug delivery to regional lymph nodes. The aim of the present study was to address biopharmaceutical factors related to the use of s.c. administered liposomes as a lymphotropic delivery system. Important factors influencing lymphatic disposition of s.c. administered liposomes were found to be the anatomical site of injection, liposome size, and the presence of phosphatidylserine (PS) in the liposomal bilayer. Other factors, such as lipid dose and the presence of a hydrophilic poly(ethyleneglycol) (PEG)-coating, had a negligible or only a slight effect on the lymphatic disposition of s.c. administered liposomes.

Keywords: Liposomes; Lymph nodes; Targeting; Subcutaneous administration

1. Introduction

Targeting of particulate carriers to the lymphatic system has a number of applications, including diagnosis and treatment of diseases with lymphatic involvement such as tumor metastases, viral and bacterial infections and immunization. The function of the lymphatic system in the clearance of excess fluid and particulates from the interstitial tissue has generated interest in the use of colloidal particles, such as liposomes, for the

Abbreviations: Chol, cholesterol; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; DSPE, diphosphatidylethanolamine; EPC, egg-phosphatidylcholine; EPG, egg-phosphatidylglycerol; i.d., intradermal; i.m., intramuscular; i.p., intraperitoneal; % ID, percentage injected dose; PEG, poly(ethyleneglycol); PS, phosphatidylserine; s.c., subcutaneous; TL, total lipids.

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delivery of diagnostic and therapeutic agents to regional lymph nodes following local administration, such as subcutaneous (s.c.), intramuscular (i.m.) and intraperitoneal (i.p.) injection (Fig. 1) (Jackson, 1981; Parker et al., 1982; Hirano and Hunt, 1985).

The s.c. route of administration has received most of the attention for lymphatic targeting of liposomes (Kaledin et al., 1982; Patel, 1988; Trubetskoy et al., 1995). However, detailed information on factors influencing lymphatic uptake and lymph node localization after s.c. administration is not readily available. Although earlier reports have demonstrated the occurrence of lymphatic uptake and lymph node localization of s.c. administered liposomes, the results obtained up to now do not provide a complete picture and are often not comparable as different liposome labels, lipid compositions and animals were used. Moreover, most studies monitored the fate of the drug rather than that of the particles.

The present paper gives an overview of the results of a systemic investigation of biopharmaceutical factors potentially influencing the lymphatic disposition of s.c. administered liposomes. First, the importance of the anatomical site of s.c. injection was studied. The relationship between physicochemical properties of the liposomes and the efficiency of lymphatic uptake and lymph node localization was studied by utilizing liposomes of various sizes and lipid compositions. The influence of steric stabilization of the liposome surface was studied by incorporation of poly(ethyleneglycol) (PEG) conjugated to distearoylphosphatidylethanolamine (DSPE) into the liposomes.

2. Materials and methods

2.1. Preparation of liposomes

Liposomes were prepared and characterized as described previously (Oussoren et al., 1997b). Briefly, liposomes were prepared by the thin film method and hydrated in a sterile Hepes/glucose buffer (10 mM Hepes, 1 mM EDTA, 270 mM glucose, pH 7.4). [^{3}H]cholesteryloleylether, which has proven to be a reliable label for monitoring the fate of liposomes in vivo, was added as a marker of the lipid phase. Liposomes were non-sized or sized by extruding the liposome dispersion through an appropriate combination of single or stacked 0.6, 0.2, 0.1 or 0.05 μm polycarbonate membrane filters under nitrogen pressure. Colloidal gold-containing liposomes were prepared as described previously (Hong et al., 1983).

2.2. Animal experiments

Rats were injected s.c. with a single dose of ^{3}H-labeled liposomes into the dorsal side (i.e. the upper side) of the right foot unless indicated otherwise. At the end of the observation period (i.e. 52 h post-injection) the site of s.c. injection and regional lymph nodes (popliteal and iliac) (Tilney, 1971) were collected and assayed for radioactivity. Radioactivity was determined as described previously (Oussoren et al., 1997b). Lymphatic uptake is defined as the percentage injected dose radioactivity (i.e. 100%) minus the percentage of dose radioactivity recovered from the injection site. Lymph node localization is ex-

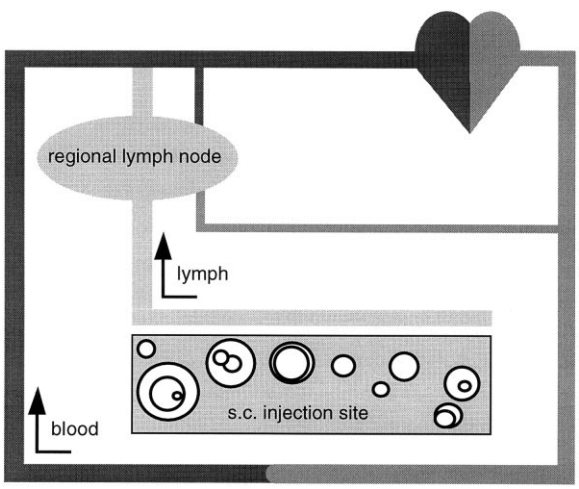

Fig. 1. Schematic representation of lymphatic absorption of s.c. injected particles. Following s.c. administration, large molecules and colloids, such as liposomes, which are too large to enter blood capillaries, can be taken up by the lymphatic system. Following lymphatic uptake, liposomes will encounter one or more lymph nodes where a fraction of the injected dose will be retained.

pressed as the percentage injected dose radioactivity per g of lymph node tissue (popliteal and iliac). Results represent the mean of four rats $\pm$ S.D.

3. Biopharmaceutical factors

3.1. Tissue structure

The influence of the tissue structure at the anatomical site of injection was studied by injection of small sized EPC:EPG:Chol (molar ratio 10:1:4) liposomes (mean size about 0.10 μm) into three different sites of the rat: the flank, the dorsal side of the foot and the footpad of rats. After s.c. administration of liposomes into the flank, lymphatic uptake from the site of injection was low ($<5\%$ of the injected dose (% ID)) and consequently, levels of radioactivity in the regional lymph nodes (i.e. axillary and brachial) were low ($<2\%$ ID/g). Lymphatic uptake from the site of injection was much higher after s.c. injection into the dorsal side of the foot or into the footpad (about 60 and 54% ID, respectively) and consequently, much higher levels of radioactivity were recovered in the regional (i.e. popliteal and iliac) lymph nodes (about 130 and 70% ID/g, respectively) (Oussoren et al., 1997a). The observed site-dependence of the lymphatic uptake process may be related to differences in the structural organization of s.c. tissue. In rats, hardly any s.c. fat layer is present at the footpad and at the dorsal side of the foot, whereas at the flank an s.c. layer of loose adipose tissue is found. As a result of the limited interstitial space at both s.c. sites of the foot, the injected dispersion may induce a rise in local interstitial pressure. In contrast to injection into the foot, s.c. injection of liposomes into the flank will not cause an increase in interstitial pressure as the injected fluid will spread over a large area of s.c. adipose tissue.

3.2. Liposome related factors

Liposome size was found to be the most important factor influencing lymphatic drainage. The influence of liposome size on lymphatic uptake and lymph node localization was studied by s.c. injection of liposomes (EPC:EPG:Chol (10:1:4)) of various sizes (0.70, 0.17 and 0.40 μm and non-sized liposomes) into the dorsal side of the foot of rats. In line with earlier findings of others, size appeared to be a crucial factor influencing lymphatic uptake of liposomes (Patel, 1988, Allen et al., 1993). Small liposomes, with a mean size of about 0.07 μm were taken up into the lymphatic capillaries to a high extent (about 62% ID), whereas large, non-sized liposomes remained almost completely at the s.c. injection site. The size-dependent uptake is likely to be related to the process of particle transport through the interstitium. The structural organization of the interstitium dictates that the diameter of administered particles should be small enough to allow migration through the aqueous channels in the interstitium. Therefore, larger, non-sized particles will have more difficulty in traversing the interstitium and will remain at the site of injection to a large, almost complete, extent (Oussoren et al., 1997b). Lymph node localization was about the same for all liposome sizes evaluated, when expressed as the % ID. However, this result should not lead to the conclusion that liposome size does not affect lymph node localization. When expressed as the percentage of the lymphatically absorbed fraction per g ('relative lymph node localization'), lymph node localization is much higher (about 8-fold) for larger liposomes than for smaller liposomes. Evidently, larger liposomes, with a size which still allows lymphatic uptake, are retained more efficiently by lymph nodes rather than smaller liposomes, possibly because larger liposomes are likely to be filtered out more efficiently in lymph nodes rather than smaller liposomes and/or, in view of the outcome of studies on the interaction between liposomes and macrophages (Senior, 1987), because larger particles may be phagocytosed more efficiently by macrophages rather than smaller liposomes.

Lipid composition was considered to be another potential factor influencing lymphatic uptake and lymph node localization of s.c. administered liposomes. Therefore, lymphatic uptake and lymph node localization of small liposomes (mean size about 0.07 μm) of several compositions (EPC, EPC:Chol, EPC:EPG,

EPC:PS, EPC:EPG:Chol, EPC:PS:Chol and DPPC:DPPG:Chol) were studied after s.c. injection into the dorsal side of the foot of rats. In general, about 40% ID remained present at the site of injection at the end of the observation period. Therefore, it was concluded that lipid composition is not a factor of importance for lymphatic uptake from the s.c. site of injection for the liposomes investigated. Also lymph node localization was not strongly affected by the liposomal lipid composition. Only phosphatidylserine (PS)-containing liposomes localized to a much higher extent (about 3-fold) in regional lymph nodes. As PS-exposure has been shown to serve as a signal for triggering recognition by macrophages, the substantially increased lymph node localization of PS-containing liposomes may be attributed to the same mechanism.

The influence of the presence of a hydrophilic steric repulsive layer on the liposomal surface was studied by inclusion of DSPE-PEG in the liposomal bilayer of small (0.07 μm) liposomes composed of EPC:Chol and DPPC:Chol. The results demonstrated that inclusion of DSPE-PEG into both types of liposomes does not influence lymphatic uptake to a high extent. Apparently, factors other than the hydrophilic steric repulsive layer on the liposomal surface are more important in determining lymphatic uptake from the s.c. injection site. Also lymph node localization was only slightly affected by DSPE-PEG-mediated steric stabilization (Oussoren et al., 1997c). The observation that the steric barrier imposed by the hydrophilic PEG-chains does not strongly suppress lymph node localization may indicate that macrophage uptake is not the only important mechanism of lymph node localization.

4. Mechanism of lymph node localization

It was observed that an increase in liposomal size and the presence of PS in the liposomal bilayers enhanced liposome localization in regional lymph nodes, confirming the hypothesis that uptake by macrophages is an important mechanism in lymph node localization of s.c. administered liposomes. However, coating of small liposomes with PEG, which has proven to slow down macrophage uptake, hardly affected the degree of lymph node localization suggesting that phagocytosis by macrophages is not the only important mechanism for lymph node localization of liposomes. The ability of clodronate-containing liposomes to deplete macrophages in lymph nodes, the so-called 'macrophage suicide-technique' (van Rooijen, and Sanders, 1994), was used as a tool to study the mechanism of lymph node localization.

Pretreatment with liposomal clodronate resulted in drastic reduction of lymph node localization of large (1 μm) as well as small (0.1 μm) liposomes (87 and 59% reduction, respectively) composed of EPC:EPG:Chol (10:1:4). However, reduction of lymph node localization of the larger liposomes was substantially higher (about 1.5-fold). These results demonstrate that macrophages play an important role in lymph node localization of liposomes. For larger liposomes, phagocytosis by lymph node macrophages is the most important mechanism. Morphologic observations on intranodal localization of s.c. administered colloid-gold containing liposomes confirmed the preferential uptake of liposomes by lymph node macrophages. Microscopic evidence was obtained which proved that small liposomes (0.1 μm) are not only taken up by macrophages but also by other cells, such as endothelial cells. Remarkably, inclusion of DSPE-PEG into the liposomes did not significantly influence lymph node localization in control and in macrophage depleted lymph nodes. Based on the present results it is concluded that liposome localization in lymph nodes is the resultant of mechanical filtration in the meshwork of the lymph node and subsequent phagocytosis by lymph node macrophages (Oussoren et al., 1998).

5. Preliminary findings on therapeutic applications

The observations of high concentrations of liposomes in regional lymph nodes were challenging to initiate studies on the therapeutic application of s.c. administered liposomes. This type of administration of liposome-encapsulated antitu-

mor drugs may prove to be a valuable adjuvant therapy for the treatment of lymphatic metastases. Doxorubicin-liposomes were used to evaluate the utility of s.c. administered liposomes on the targeting of antitumor agents to regional lymph nodes bearing tumor metastases. The effect of liposome encapsulation on lymphatic uptake and lymph node localization of doxorubicin was studied in the Line 10 guinea pig model, which is a hepatocellular carcinoma characterized by progressive growth of a local tumor at the inoculation site and fast development of regional metastases in lymph nodes.

Preliminary results demonstrate that liposomal doxorubicin (five injections of 0.2 mg on consecutive days) reduces growth of lymph node metastasis completely. Despite enhanced drug disposition in the metastatic lymph nodes, liposomal doxorubicin was found to be equally effective as free doxorubicin. However, a major advantage of liposomal doxorubicin, in particular at higher (cumulative) doses, would be a reduction of local tissue toxicity.

6. Conclusions

Decisive factors influencing lymphatic disposition of s.c. administered liposomes are the tissue structure at the s.c. site of injection and liposomal size. Liposomes s.c. injected either into the dorsal side of the foot or into the footpad of rats are absorbed from the injection site and localize in regional lymph nodes to a high extent, whereas liposomes injected s.c. into the flank of rats are hardly absorbed from the site of injection and do not reach regional lymph nodes to a significant extent. Liposome size is the most important liposome related factor influencing lymphatic uptake of s.c. administered liposomes. Small liposomes are taken up from the s.c. injection site to a great extent, whereas larger liposomes predominantly remain at the injection site. Lymph node localization was substantially enhanced by inclusion of PS into the liposomal bilayers. Relative lymph node localization was found to be relatively high for larger liposomes, suggesting more efficient intranodal capturing of larger liposomes. Other factors such as lipid composition, liposomal surface charge and the presence of a hydrophilic coating do not substantially affect lymphatic disposition of s.c. administered liposomes.

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