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Short communication

Role of macrophages in the localisation of liposomes in lymph nodes after subcutaneous administration

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Abstract

The macrophage 'suicide' technique, based on the ability of clodronate-containing liposomes to deplete lymph nodes of macrophages, was used to study the role of macrophages in lymph node localisation of subcutaneous (s.c.) administered liposomes. Reduced liposome localisation in macrophage depleted lymph nodes confirmed that phagocytosis by macrophages is an important mechanism for lymph node localisation of large (non-sized) liposomes. Depletion of macrophages had less effect on the lymph node localisation of small (about 0.1 μm) liposomes; small liposomes may reach macrophages in regions of lymph nodes not reached by large liposomes. Small liposomes may also be taken up by cells other than macrophages, such as endothelial cells lining the lymph node sinuses. Remarkably, inclusion of poly(ethyleneglycol)-distearoylethanolamine (PEG-PE) into liposomes did not reduce the degree of lymph node localisation in control lymph nodes. As macrophage depletion had a strong negative effect on the lymph node localisation of PEG-liposomes, it is concluded that, despite the presence of a PEG-coating, PEG-liposomes retained by lymph nodes are to a large extent taken up by lymph node macrophages.

Keywords: Liposomes; Lymph nodes; Localisation; Subcutaneous administration

Abbreviations: Chol, cholesterol; DSPE, distearoylphosphatidylethanolamine; EPC, egg-phosphatidylcholine; EPG, egg-phosphatidylglycerol; PEG-PE, poly(ethyleneglycol)-distearoylethanolamine; s.c., subcutaneous; TL, total lipid.

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Following interstitial administration, large molecules and colloids which are too large to enter blood capillaries, can be absorbed via the lymphatic system. The ability of the lymphatics to take up colloids from interstitial spaces has been exploited for the targeting of drug carriers into the lymphatic system. In particular liposomes, being versatile, well tolerated, biodegradable lipid vesicles (Storm et al., 1993), have received considerable interest as vehicles for drug targeting to the lymphatic system. Subcutaneous (s.c.) injection has been the route of administration most extensively investigated for this purpose.

After s.c. injection of liposomes into the dorsal side of the foot of rats, liposomes are efficiently absorbed via lymphatic capillaries draining the site of injection (Oussoren et al., 1997). Once liposomes have entered the lymphatic capillaries, they pass through the lymphatic system where they can be captured in regional lymph nodes. Uptake of s.c. administered liposomes in regional lymph nodes (popliteal and iliac) may be up to 30–40-fold higher than uptake in liver and spleen (Fig. 1). Considering the fact that liver and spleen are the natural targets for circulating liposomes, uptake of liposomes by lymph nodes is apparently an efficient process. It has been suggested that

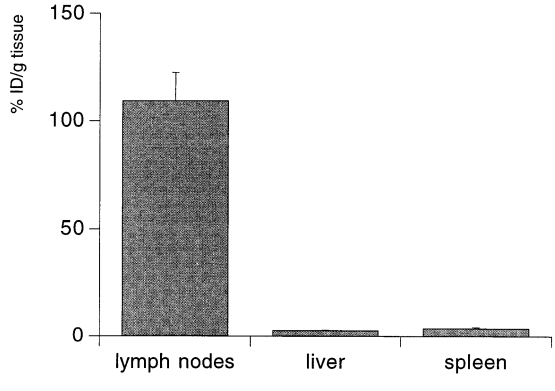

Fig. 1. Biodistribution of liposomes after subcutaneous (s.c.) administration. A single dose of liposomes [egg-phosphatidylcholine (EPC):egg-phosphatidylglycerol (EPG):cholesterol (Chol), 10:1:4, molar ratio, mean diameter 0.1 μm, 2.5 μmol total lipid (TL)] was injected s.c. into the dorsal side of the foot of rats. Comparison of the percentage of injected dose per gram tissue in regional lymph nodes, liver and spleen. Values represent the mean percentage ± S.D. of four animals.

phagocytosis by macrophages is one of the major mechanisms of uptake of colloidal particles in lymph nodes (O'Hagan et al., 1992; Velinova et al., 1996). Recently, it was observed that the presence of phosphatidylserine in the liposomal bilayers substantially enhanced lymph node localisation, supporting the hypothesis that macrophage uptake is the major mechanism involved in lymph node localisation of liposomes after s.c. administration (Oussoren et al., 1997). However, on the other hand, coating of small (0.1 μm) liposomes with poly(ethyleneglycol) (PEG), which has proven to oppose macrophage uptake (Allen 1994), hardly affected lymph node localisation, suggesting that phagocytosis by macrophages is not the only mechanism involved in lymph node retention of liposomes (Oussoren and Storm, 1997).

The main objective of the present study was to gain more insight into the role of macrophages in the localisation of s.c. administered liposomes in regional lymph nodes. The ability of clodronate-containing liposomes to deplete macrophages in lymph nodes was used as a tool to investigate the localisation of intranodal liposomes (Van Rooijen and Sanders, 1994). Macrophages in the popliteal lymph node were depleted by s.c. administration of clodronate-containing liposomes. S.c. injection of clodronate-liposomes results in depletion of macrophages lining the subcapsular sinus and those in the medulla of popliteal lymph nodes (Delemarre et al., 1990). Six days after s.c. administration of clodronate-liposomes, liposomes labelled with [3H]cholesteryloleylether were injected s.c. into the dorsal side of the right (pretreated) and left (control) foot. Localisation of s.c. administered large (non-sized) and small (0.1 μm) liposomes composed of egg-phosphatidylcholine (EPC):egg-phosphatidylglycerol (EPG):cholesterol (Chol) (molar ratio 10:1:4) was studied in the popliteal lymph node depleted of macrophages and compared with localisation in control (non-depleted) lymph nodes 24 h post-injection.

Pretreatment with liposomal clodronate resulted in drastic reduction of lymph node localisation of large as well as small liposomes (Fig. 2). However, the reduction in lymph node localisation was substantially higher (about 1.5-fold) in

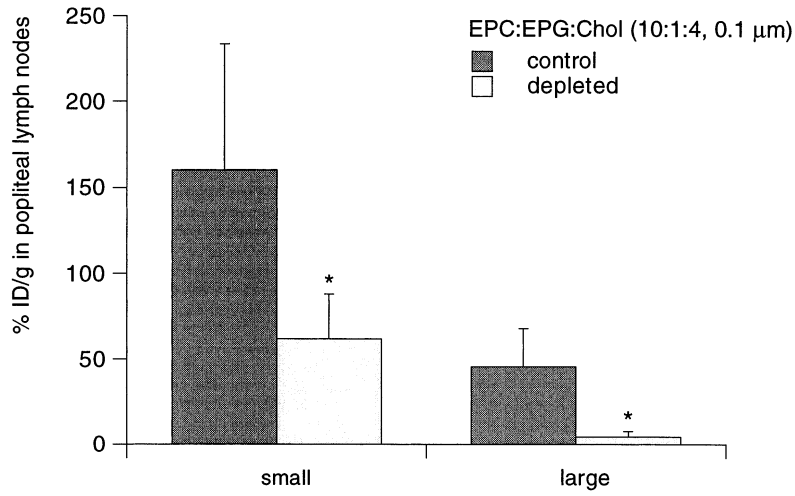

Fig. 2. Effect of depletion of lymph node macrophages on lymph node localisation of subcutaneous (s.c.) administered liposomes. Macrophages in regional lymph nodes were eliminated by s.c. injection of clodronate-liposomes. Six days after pretreatment rats were s.c. injected with radiolabelled liposomes [egg-phosphatidylcholine (EPC):egg-phosphatidylglycerol (EPG):cholesterol (Chol), 10:1:4, molar ratio, mean diameter 0.1 μm, 2.5 μmol total lipid (TL)] into the right foot (pretreated with liposomal clodronate) and left foot (control). Localisation of liposomal label was determined 24 h post-injection. (* $P < 0.05$).

the case of the large liposomes compared to the small liposomes. The difference in degree of lymph node localisation in macrophage-depleted lymph nodes between small and large liposomes may be related to the ability of small liposomes to penetrate more efficiently into the nodal tissue than large liposomes. As macrophages in the subcapsular sinus and medulla were completely depleted, clodronate-liposomes are probably phagocytosed predominantly by macrophages present in these lymph node areas. The large liposomes will likely follow the routing of the large, clodronate-liposomes through the lymph node and, therefore, tend to pass through lymph node regions which are depleted from macrophages. Consequently, phagocytosis of these large liposomes will be strongly reduced. Considering the finding that lymph node retention is also strongly reduced, the involvement of macrophages for lymph node localisation of large liposomes is clearly indicated. Small liposomes, as a result of their size, might be able to penetrate more efficiently into deeper areas of the lymph node and may, therefore, reach lymph node regions not encountered by the large clodronate-liposomes. Thus, phagocytosis of small liposomes may still occur by macrophages present in non-depleted areas of the lymph node. A second explanation for the difference in effect of liposomal clodronate-treatment on lymph node localisation between large and small liposomes relates to the possibility of uptake of small liposomes by cells other than macrophages. Small liposomes may be taken up by cells, such as endothelial cells, capable of pinocytosis of macromolecules and very small particles (Hoefsmit et al., 1980; Lázár et al., 1989).

In order to visualise the liposomes in regional lymph nodes, liposomes containing colloidal gold were prepared (as described by Hong et al., 1983) and their localisation in regional lymph nodes was examined by light microscopy and transmission electron microscopy. Morphological observations visualising the uptake of liposomes by lymph nodes confirmed macrophages as the predominant site of liposome localisation. In all cases, colloidal gold was found in intracellular vesicles. In the case of injection of small liposomes, colloidal gold was also observed in endothelial cells lining the lymphatic sinuses (Oussoren et al., 1998).

Sterically stabilised small (0.1 μm) and large (non-sized) liposomes containing the lipid deriva-

tive distearoylphosphatidylethanolamine-PEG2000 (DSPE-PE) and composed of EPC:EPG:Chol:PEG-PE (molar ratio 10:1:4:1) (PEG-liposomes) were also included in this study. As surface modification with PEG is known to resist phagocytosis of liposomes by macrophages, it was anticipated that lymph node localisation of PEG-liposomes would be substantially lower as compared to liposomes lacking the PEG-coating. Moreover, one would expect that the depletion of macrophages has only a minor effect on lymph node localisation of PEG-liposomes. However, it was observed that inclusion of PEG-PE did not substantially influence localisation in control lymph nodes. In addition, macrophage depletion had a similar effect on the degree of localisation of PEG-liposomes as for liposomes lacking the PEG-coating (Fig. 3). Remarkably, also morphological observations confirmed the localisation of PEG-liposomes within the macrophages. These results can not be attributed to a failure to achieve effective steric stabilisation, as the PEG-liposomes proved to be long-circulating after reaching the blood circulation (Oussoren and Storm, 1997). It is hypothesised that the initial mechanism of lymph node localisation may be the result of mechanical depth filtration in the meshwork of reticular cells in the lymph node. Because of the slow progression of the liposomes through the lymph node, enough time might be available for an effective interaction of the PEG-liposomal surface with phagocytic cell membranes and, given enough time, even PEG-liposomes will be taken up by macrophages. In this context, one should realise that it is the rate rather than the extent of phagocytosis that is affected by the hydrophilic PEG-coating; macrophages of liver and spleen still ingest a substantial part of an i.v. dose of PEG-liposomes. Partial dissociation of the steric barrier from the particle surface has been suggested to be responsible for the observed uptake of poloxamine-908 coated polystyrene particles by spleen macrophages (Moghimi 1995; Storm et al., 1995). However, in the view of the relatively early observation time point (6 h post-injection) and the much stronger anchoring of PEG to the liposomal bilayers via the hydrophobic anchor DSPE as compared to the physically adsorbed poloxamine-908, this possibility seems unlikely. The reason for the apparent efficient

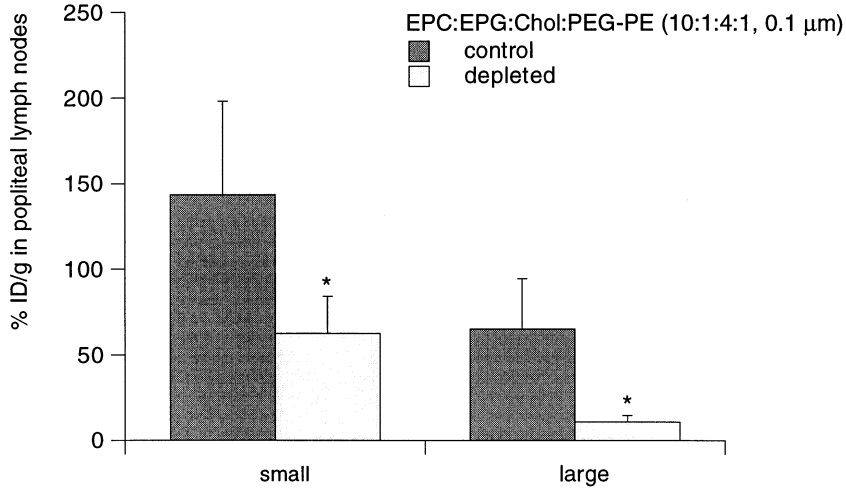

Fig. 3. Effect of depletion of lymph node macrophages on lymph node localisation of subcutaneous (s.c.) administered poly(ethyleneglycol (PEG)-liposomes. Macrophages in regional lymph nodes were eliminated by s.c. injection of clodronate-liposomes. Six days after pretreatment rats were s.c. injected with radiolabeled liposomes [egg-phosphatidylcholine (EPC):egg-phosphatidylglycerol (EPG):cholesterol (Chol), 10:1:4:1, molar ratio, mean diameter 0.1 μm, 2.5 μmol total lipid (TL)] into the right foot (pretreated with liposomal clodronate) and left foot (control). Localisation of liposomal label was determined 24 h post-injection. (* $P < 0.05$).

uptake of PEG-liposomes by lymph node macrophages remains to be investigated. Observations on earlier time-points after injection might reveal differences between the localisation of control and PEG-liposomes.

In conclusion, reduced lymph node localisation of liposomes in macrophage-depleted lymph nodes confirmed that phagocytosis by macrophages plays an important role in nodal retention of liposomes. For large, non sized liposomes phagocytosis by macrophages is the most important mechanism of lymph node localisation. Small (0.1 μm) liposomes may reach macrophages in regions of the lymph node not reached by large liposomes and might be taken up by cells other than macrophages, such as endothelial cells. Inclusion of PEG-PE into the liposomes, which is known to oppose macrophage uptake, did not affect lymph node localisation in macrophage-depleted or control lymph nodes. Apparently, PEG-liposomes retained by lymph nodes are also taken up by lymph node macrophages. Morphological observations visualising the uptake of PEG-liposomes by lymph node macrophages support this conclusion.

References

Allen, T.M., 1994. The use of glycolipids and hydrophilic polymers in avoiding rapid uptake of liposomes by the mononuclear phagocyte system. Adv. Drug Deliv. Rev. 13, 285–309.

Delemarre, F.G.A., Kors, N., Kraal, G., Van Rooijen, N., 1990. Repopulation of macrophages in popliteal lymph nodes of mice after liposome-mediated depletion. J. Leukocyte Biol. 47, 251–257.

Hoefsmit, E.C.M., Kamperdijk, E.W.A., Hendricks, H.R., Beelen, R.H.J., Balfour, B.M., 1980. Lymph node macrophages. In: Carr, I., Daems, W.T. (Eds.), The Reticuloendothelial System, vol. I. Plenum, New York, pp. 417–468.

Hong, H., Friend, D.S., Glabe, C.G., Papahadjopoulos, D., 1983. Liposomes containing colloidal gold are a useful probe of liposome cell-interactions. Biochim. Biophys. Acta 732, 320–323.

Lázár, G., Van Galen, M., Scherphof, G.L., 1989. Gadolinium chloride-induced shifts in intrahepatic distributions of liposomes. Biochim. Biophys. Acta 1011, 87–101.

Moghimi, S.M., 1995. Mechanisms of splenic clearance of blood cells and particles: towards development of new splenotropic agents. Adv. Drug Deliv. Rev. 17, 103–115.

O'Hagan, D.T., Christy, N.M., Davis, S.S., 1992. Particulates and lymphatic drug delivery. In: Charman, W.N., Stella, V.J. (Eds.), Lymphatic Transport of Drugs. CRC press, Boca Raton, pp. 279–315.

Oussoren, C., Zuidema, J., Crommelin, D.J.A., Storm, G., 1997. Lymphatic uptake and biodistribution of liposomes after subcutaneous injection. II. Influence of liposomal size, lipid composition and lipid dose. Biochim. Biophys. Acta 1328, 261–272.

Oussoren, C., Storm, G., 1997. Lymphatic uptake and biodistribution of liposomes after subcutaneous injection. III. Influence of surface modification with poly(ethyleneglycol). Pharm. Res. 14, 1479–1484.

Oussoren, C., Velinova, M., Scherphof, G., van der Want, J.J., van Rooijen, N., Storm, G., 1998. Lymphatic uptake and biodistribution of liposomes after subcutaneous injection. IV Fate of liposomes in regional lymph nodes. Biochim. Biophys. Acta, 1370, 259–272.

Storm, G., Oussoren, C., Peeters, P.A.M., Barenholz, Y., 1993. Tolerability of liposomes in vivo. In: Gregoriadis, G. (Ed.), Liposome Technology, vol. III. CRC press, Boca Raton, pp. 345–383.

Storm, G., Belliot, S.O., Daemen, T., Lasic, D.D., 1995. Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. Adv. Drug Deliv. Rev. 17, 31–48.

Van Rooijen, N., Sanders, A., 1994. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. J. Immunol. Methods 174, 83–93.

Velinova, M., Read, N., Kirby, C., Gregoriadis, G., 1996. Morphological observations on the fate of liposomes in the regional lymph nodes after footpad injection into rats. Biochim. Biophys. Acta 1299, 207–215.