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Subcutaneous administration of liposomes: a comparison with the intravenous and intraperitoneal routes of injection

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The development of long-circulating liposomes containing lipid derivatives of poly(ethylene glycol) (PEG), termed Stealth® liposomes, has considerably improved the prospects for therapeutic applications of liposomal drug delivery systems. We have examined the pharmacokinetics and biodistribution of long-circulating, as compared to conventional, liposomes after subcutaneous (sc) administration in mice. Results obtained after subcutaneous administration were compared to those obtained after intravenous (iv) and intraperitoneal (ip) administration. Liposomes, following sc administration, appeared intact in the circulation subsequent to moving down the lymph node chains that drain the site of injection. Liposomes containing PEG-distearoylphosphatidylethanolamine (PEG-DSPE) resulted in the highest levels of small (80–90 nm) liposomes in the blood, with up to 30% of in vivo label appearing in the blood at 12 to 24 h post-injection. In the absence PEG-DSPE approx. 4-fold lower levels of liposomes were found in the blood. Small size of the liposomes was critical to their ability to move into the circulation, with liposomes above 110–120 nm not appearing in blood to any significant extent. The presence of PEG-DSPE and cholesterol was important for the in vivo stability of the liposome after sc administration. Although liposome levels were significantly higher in the draining lymph nodes after sc administration, levels associated with other tissues were proportionately reduced relative to the iv and ip routes of administration. Liposomes appeared in blood after ip and sc administration with half-lives of approx. 0.6 and 9 h, respectively, and subsequent to appearing in blood had similar biodistribution, pharmacokinetics and half-lives (20.4 h) to liposomes given by the iv route.

Introduction

New long-circulating formulations of liposomes, which contain lipid derivatives of poly(ethylene glycol), are beginning to achieve widespread acceptance as drug delivery systems [1–6]. These liposomes have been termed Stealth® [1], or sterically-stabilized liposomes (S-liposomes) for their ability to avoid detection by the mononuclear phagocyte system (MPS) in vivo [1,7]. Rapid uptake of liposomes by the MPS, and saturation of this important host defense system, has been a significant drawback to liposome formulations used in past studies (reviewed in Ref. 8). Besides their long circulation half-lives, S-liposomes have the additional advantage of dose-independent pharmacokinetics as a result of their lack of saturation of the MPS in mice at doses as high as 400 mg phospholipid/kg [9].

Several studies have examined the pharmacokinetics and/or biodistribution of S-liposomes after administration by the intravenous (iv) and intraperitoneal (ip) routes [9–13]. Although useful in animal models of disease, the ip route is unlikely to be of much use in human therapy except in specialized circumstances, and the iv route requires a health professional for administration. We were interested in examining the subcutaneous (sc) route of administration of liposomes, not only because it is a simpler route for patient self-administration, but also because it might serve as a depot for the sustained release of drug in vivo. In this study we have compared sc administration of S-liposomes with conventional liposome formulations as a function of liposome dose, size, phospholipid phase transition and the presence or absence of cholesterol.

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[1] Stealth is a registered trademark of Liposome Technology Inc., Menlo Park, CA, USA.

Materials and Methods

Materials

Hydrogenated soy phosphatidylcholine (HSPC), partially hydrogenated phosphatidylcholine with an iodine number of 40 (PC40) [14] and poly(ethylene glycol) (average molecular mass of 1900 daltons) covalently linked via a carbamate bond to distearoylphosphatidylethanolamine (PEG-DSPE) were generous gifts of Liposome Technology Inc. (Menlo Park, CA). The synthesis of PEG-DSPE has been described previously [11]. Phosphatidylglycerol (PG) was purchased from Avanti Polar Lipids (Birmingham, AL). Cholesterol (CHOL) and *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Tes) were purchased from Sigma (St Louis, MO). Na^{125}I was purchased from the Edmonton Radiopharmaceutical Center. Tyraminylinulin was synthesized and ^{125}I-tyraminylinulin (^{125}I-TI) was prepared according to the technique of Sommerman et al. [15]. Pyrogen-free saline (0.9% USP) was obtained from Travenol Canada (Mississauga, Ontario).

Liposome preparation

Liposomes were extruded multilamellar vesicles (MLV) composed of HSPC or PC(40), with or without CHOL at a 2:1 molar ratio (phospholipid to CHOL) and containing 5 mol% of either PEG-DSPE or PG. PG was substituted for PEG-DSPE in control liposome preparations in order to result in liposomes with the same overall net negative charge. Liposomes were prepared by vortexing dried lipid films in 10 mM Tes-buffered saline, 154 mM NaCl, pH 7.4 (buffer), containing ^{125}I-TI as an aqueous space label. Liposomes were extruded through two stacked Nuclepore filters from 1.0 to 0.05 μm in pore diameter [16,17]. The resulting liposomes were sized by dynamic light scattering using a Brookhaven BI-90 particle sizer (Brookhaven Instruments, Holtsville, NY). Liposome size ranged from 79 to 93 nm for vesicles extruded through 0.05 μm filters and 328–718 nm for vesicles extruded through 1.0 μm filters. Free ^{125}I-TI was separated from entrapped label by chromatography over an Ultragel AcA34 column (IBF Biotechnics, France). For doses of 10 μmol per mouse, in order to separate free from entrapped label, the liposomes were washed three times with buffer by centrifugation at 360 000 $\times g$ for 3 h for small liposomes and 1 h for large liposomes. Size distributions were checked before and after the centrifugation step. The average size of the liposomes increased by less than 10% which was not considered significant. For liposomes below 120 nm the polydispersity was low and the liposome preparations appeared as sharp peaks by quasielastic light scattering with an average range of $\pm$25 nm. Polydispersity increased rapidly for liposomes of average diameter of 150 nm and above. Phospholipid concentrations were determined by the method of Bartlett [18].

Biodistribution studies

Female CD$_1$(ICR)BR (outbred) mice in the weight range of 23–30 g were obtained from Charles River Canada (St. Constant, Que), and maintained in standard housing. Mice (three per group) were given a single bolus injection with 0.2 ml of liposomes containing approx. 10^6 ^{125}I-TI cpm and either 0.5 or 10 μmol phospholipid. Some groups of mice received injections of free ^{125}I-TI. Injections were either subcutaneous (in the neck or in the upper back just below the neck), intravenous (via the tail vein) or intraperitoneal. After specified periods of time, animals were anaesthetized with halothane (M.T.C. Pharmaceutical, Ontario) and killed by cervical dislocation. Samples of blood (0.1 ml) and internal organs (liver, spleen, lung, kidney, heart, thyroid and carcass, which was the remainder of the animal) were collected, tissues were washed and blotted dry to remove any superficial blood and counted for label in a Beckman 8000 gamma counter. Blood correction factors, having previously been determined from ^{111}In-labelled red blood cells [7], were applied to tissues and carcass. In some experiments, heparinized blood was collected and blood cells were separated from plasma by 2 min centrifugations in a Microcentrifuge (Model 235B). The cells were washed twice with 1 ml 10 mM Tes-buffered saline and the radioactivity associated with cells or plasma plus washes were counted for ^{125}I-TI. ^{125}I-TI is metabolically inert and eliminated rapidly by filtration through the kidneys upon release from liposomes; therefore radiolabel in blood represents intact circulating liposomes and radiolabel in tissues represents liposomes which have been taken up into cells [15,19]. The data is presented as % of in vivo cpm, which represents the % of counts remaining in the body at a given time point. This corrects for leakage of the label from the liposomes and represents intact liposomes remaining in the body. The extent of leakage of label from the circulating liposomes was calculated as the ratio of cpm which remain in the whole animal to injected cpm.

In some experiments additional tissues, including bone marrow and lymph nodes were sampled, as previously described [20]. Results from these experiments are expressed as cpm/mg tissues normalized to 10^6 injected cpm and give the relative concentrations of liposomes in each tissue sampled.

Pharmacokinetics

All pharmacokinetic parameters were calculated from the curve fitting program RSTRIP (Micromath, Salt Lake City, USA). Areas under the time versus concentration curves (AUC) were extrapolated from zero to the last time point, normally 384 h (16 days), or

where blood levels for all liposome compositions had reached zero.

Results

Following sc administration, radiolabelled liposomes could be found in the blood with peak levels occurring between 6 and 24 h post-injection. When the free ^{125}I-TI label was injected sc, it was rapidly eliminated from the blood with a half-life of 7.5 min, i.e., less than 0.1% of injected cpm remaining in blood at 12 h post-injection and 2% remained in carcass (Table I). Therefore the radiolabel detected in blood was liposome-entrapped. Peak blood levels of liposomes varied over a large range (Fig. 1A), depending on the presence or absence of PEG-DSPE or cholesterol, and liposome size and dose. Small liposomes (80–90 nm in diameter) containing PEG-DSPE resulted in the highest blood levels (Fig. 1A). When PG was substituted for PEG-DSPE significantly lower blood levels of liposomes were obtained (Fig. 1A). Fig. 1B compares, as a function of liposome composition, the % of injected cpm remaining in the whole animal at 16 days post-injection, which may provide a measure of liposome stability. It is apparent that liposomes containing PEG-DSPE and CHOL resulted in the highest levels of ^{125}I-TI counts remaining in vivo, while liposomes lacking PEG-DSPE and CHOL resulted in the lowest in vivo levels of ^{125}I-TI at 16 days post-injection. There was a tendency for the liposomes given at the higher dose of 10 μmol/mouse to result in higher in vivo cpm at 16 days post-injection than that seen for liposomes given at the lower dose of 0.5 μmol/mouse, but the results for ^{125}I-TI cpm at 16 days post-injection did not vary in any significant way with liposome size (Fig. 1B).

Significant trends in blood levels in Fig. 1A, as a function of liposome size, dose and composition, have been presented as a function of time in Fig. 2. Comparison of blood levels of small liposomes, with or without PEG-DSPE, is given in Fig. 2A. The presence of PEG-DSPE resulted in significantly higher blood levels of liposomes, independent of whether the liposomes were made of saturated (HSPC) or unsaturated (PC40) phospholipids. The presence of cholesterol in the liposomes resulted in higher blood levels of liposomes then when it was absent, both for HSPC-containing liposomes (Fig. 2B) and PC40-containing liposomes, where the results were very similar (not shown). When large liposomes (328–718 nm in diameter) were injected sc, blood levels were significantly lower than when small liposomes were injected (Fig. 2C), and liposomes given at high doses (10 μmol phospholipid/mouse) resulted in higher blood levels than when given at low doses (0.5 μmol/mouse) (Fig. 2C). When blood was taken from

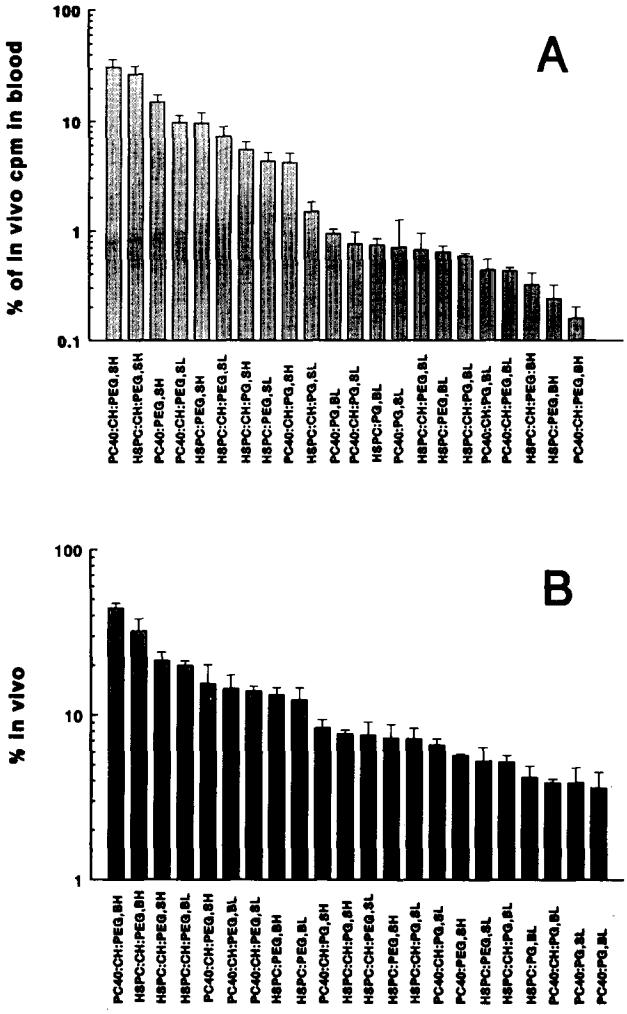

Fig. 1. Mice (three per group) were injected sc in the back of the neck with single doses of various compositions of liposomes, either low (L) dose (0.5 μmol PL/mouse) or high (H) dose (10 μmol PL/mouse). Liposomes were either small (S), with diameters of 79–93 nm, or big (B), with diameters of 328–718 nm. Liposomes were labelled with ^{125}I-TI. (A) Peak blood levels expressed as % of in vivo cpm ± S.D. (B) % of injected cpm remaining in vivo at 16 days post-injection ± S.D.

TABLE I

Levels of free ^{125}I-tyraminylinulin in blood and carcass at various times after subcutaneous administration in the back of the neck in mice (three per group)

Results are expressed as % of injected cpm ± S.D.

Time post-injection (h)	% of injected free ^{125}I-tyraminylinulin (cpm ± S.D.)	
	blood	carcass
0.5	3.7 ± 0.4	45.4 ± 3.3
1	2.1 ± 0.3	39.6 ± 12.9
2	0.9 ± 0.3	13.8 ± 2.4
4	0.4 ± 0.1	6.6 ± 1.4
6	0.1 ± 0.0	3.4 ± 1.0
12	0.1 ± 0.0	2.1 ± 0.3
24	0.1 ± 0.1	1.9 ± 0.7
48	0.1 ± 0.0	1.6 ± 0.2

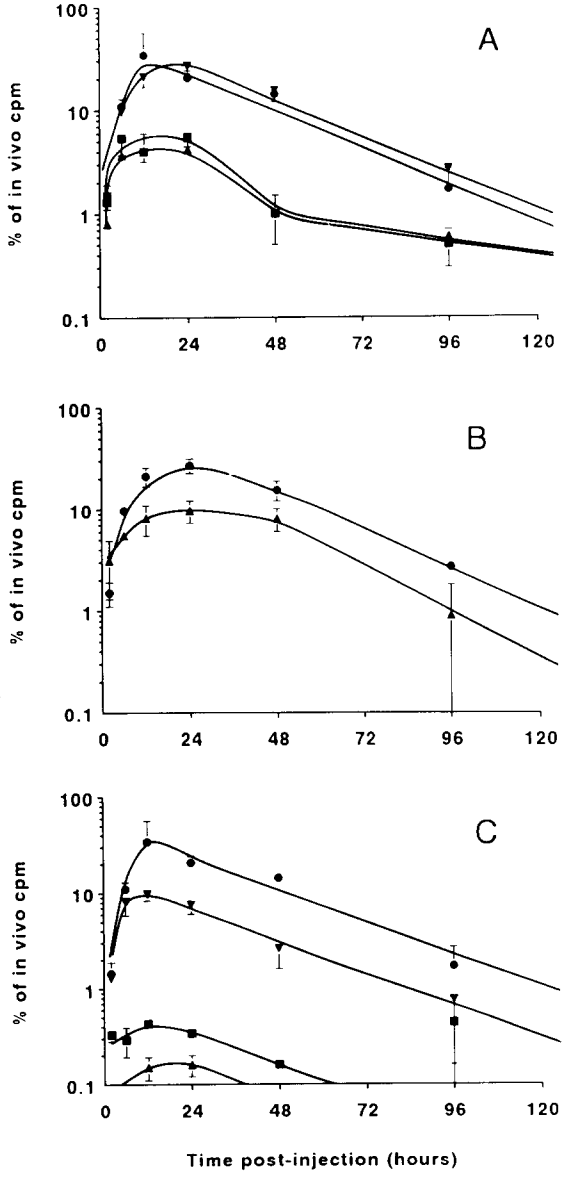

Fig. 2. Blood levels in mice (three per group) for various compositions, sizes and doses of liposomes as a function of time post-injection. Liposomes were labelled with ^{125}I-TI and results are expressed as % of in vivo cpm ± S.D. (A) Liposomes were given at a dose of 10 μmol/mouse. HSPC/CHOL/PEG-DSPE, average diameter of 89 nm (▼); PC40/CHOL/PEG-DSPE, 79 nm (●); HSPC/CHOL/PG, 74 nm (■); PC40/CHOL/PG, 90 nm (▲). (B) Liposomes were given at a dose of 10 μmol/mouse. HSPC/CHOL/PEG-DSPE, 89 nm (●); HSPC/PEG-DSPE, 83 nm (▲). (C) Liposomes were composed of PC40/CHOL/PEG-DSPE. (●) 10 μmol/mouse, 79 nm; (▼) 0.5 μmol/mouse, 82 nm; (■) 10 μmol/mouse, 460 nm; (▲) 0.5 μmol/mouse, 656 nm.

mice at times corresponding to peak blood levels (12–24 h post-injection) and separated into cellular and plasma fractions, the liposomes were present exclusively (> 99%) in the plasma fraction for liposomes with or without PEG-DSPE (not shown).

Fig. 3 shows, for HSPC/CHOL/PEG-DSPE liposomes, the effect of average liposome diameter on the peak blood levels. It can be seen that there is a

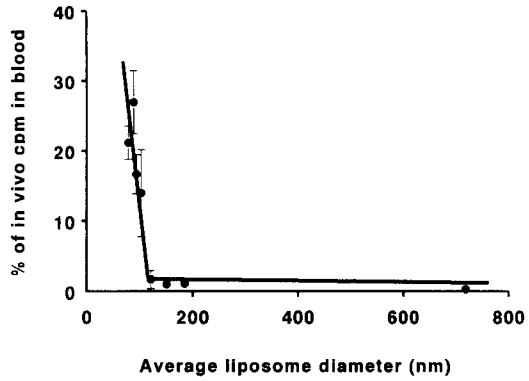

Fig. 3. Maximum blood levels (at 12 or 24 h post-injection) of liposomes (HSPC/CHOL/PEG-DSPE, 2:1:1) in mice (three per group) as a function of average liposome diameter. Results are expressed as % of in vivo cpm ± S.D.

dramatic reduction in the levels of liposomes in blood as the size of the liposomes increases. There appears to be a cut-off of approx. 120 nm in diameter above which the liposomes fail to appear in blood to any significant extent. This suggests that the liposomes may be entering the blood, via the lymphatic circulation (see below) through a 'pore' of defined size.

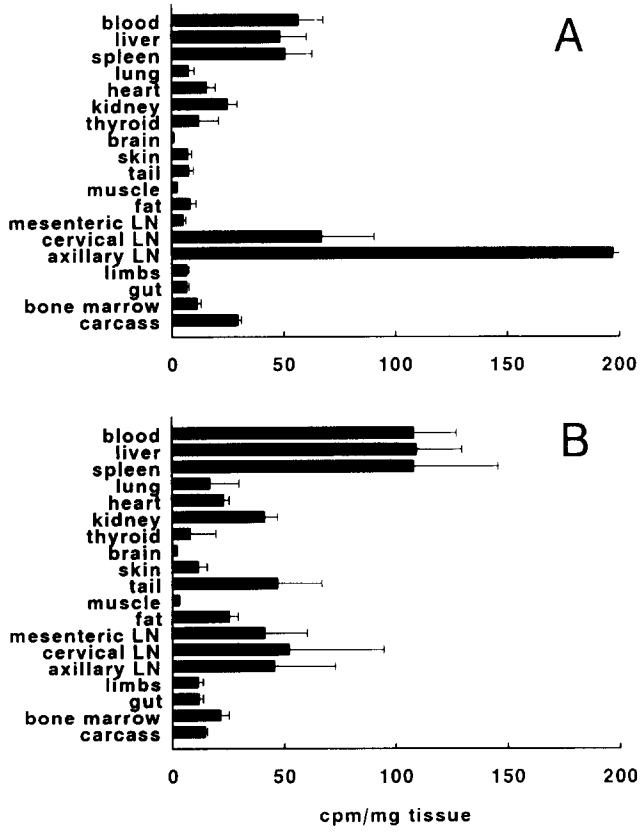

Fig. 4. Tissue distribution in mice (three per group) at 48 h post-injection of PC40/CHOL/PEG-DSPE liposomes (76 nm, 10 μmol/mouse) by the sc route (A), or the iv route (B). Results are expressed as cpm/mg tissue ± S.D., normalized to 10^6 cpm of ^{125}I-TI injected/mouse. Axillary and brachial lymph nodes are included together under the caption axillary LN.

The tissue distribution of PC40/CHOL/PEG-DSPE liposomes at 48 h following sc administration is shown in Fig. 4A, and Fig. 4B shows their tissue distribution following iv administration for comparison. Following both routes of administration, the distribution of liposomes is widespread throughout the body, and the concentration of liposomes is lower following sc administration than following iv administration in all tissues except in remaining carcass (which contains the site of sc injection), and in the cervical, and axillary and brachial lymph nodes, which drain the region of the injection.

When the distribution of labelled liposomes (PC40/CHOL/PEG-DSPE, 90 nm) was examined in the mesenteric, cervical, combined axillary and brachial lymph nodes, and blood as a function of time after sc administration it can be seen that the mesenteric lymph nodes have low concentrations of liposomes relative to the other two sets of lymph nodes (Fig. 5A). Concentrations of liposomes associated with cervical nodes and with axillary and brachial nodes tended to increase initially and then gradually decreased with time post-injection (Fig. 5A). A similar trend was observed in blood levels of liposomes with time. This suggests that small S-liposomes are reaching blood by moving down the lymph node chains which drain the site of injection.

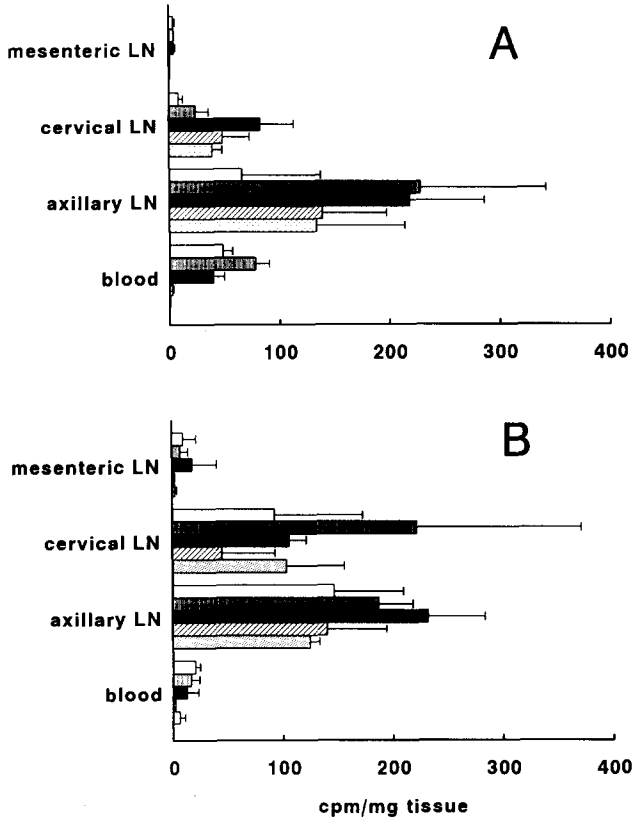

Fig. 5. Lymph node levels of liposomes as a function of time post-injection. Mice received either (A) PC40/CHOL/PEG-DSPE liposomes (90 nm, 10 μmol PL/mouse) or (B) PC40/CHOL/PG liposomes (87 nm, 10 μmol PL/mouse) by the sc route of injection. Results are expressed as cpm/mg tissue $\pm$ S.D., normalized to 10^6 cpm of ^{125}I-TI injected/mouse. Open bars, 12 h post-injection; shaded bars, 24 h; solid bars, 48 h; cross-hatched bars, 96 h; stippled bars, 192 h post-injection.

For large liposomes in the presence or absence of PEG (PC40/CH/PEG-DSPE, 665 nm or PC40/CH/PG, 444 nm) the levels of liposomes in all sampled lymph nodes and in blood were negligible (< 5 cpm/mg and usually < 1 cpm/mg) suggesting that the large liposomes were not leaving the site of injection to any significant degree (not shown). In the absence of PEG-DSPE, sc injection of small liposomes (PC40/CH/PG, 87 nm) resulted in higher levels of liposomes in lymph nodes, particularly at early times points, and lower blood levels (Fig. 5B). This suggests that, although the liposomes were leaving the site of injection they were being taken up by macrophages or monocytes in lymph nodes to a greater degree than small liposomes containing PEG-DSPE.

Fig. 6 provides a comparison between the iv, ip and sc routes of injection, as a function of time, for blood (Fig. 6A), liver (Fig. 6B), spleen (Fig. 6C), and remaining carcass (Fig. 6D) for liposomes composed of PC40/CHOL/PEG-DSPE (2 : 1 : 0.1, molar ratio). Blood levels, after iv injection, followed log-linear pharmacokinetics, as has been previously described [9]. Following both ip and sc injection blood levels initially rose as the liposomes found their way into the blood, and then fell as the liposomes were eliminated from the blood with similar pharmacokinetics as that seen following iv administration of liposomes (Fig. 6A). Blood levels were highest following iv administration, but following ip administration, blood levels approaching iv administration were achieved. Lower blood levels were found after sc administration of liposomes, however, even after sc administration peak blood levels of 30% of in vivo cpm could be achieved (Fig. 1A, Fig. 6A). The principal tissues of liposome uptake were liver and spleen, with levels of uptake being proportional to blood levels following each route of administration, i.e., lower blood levels, and very low liver and spleen levels of liposomes were observed following sc, as compared to iv or ip, administration. Indeed, following sc administration of liposomes, liver levels remained below 10% and spleen levels below 1% of in vivo cpm (Fig. 6B, 6C). Because the carcass contains liposomes residing at the site of ip and sc injection, residual carcass levels initially fell as liposomes were released into blood, and then increased as the liposomes found their way via the bloodstream to other tissues (Fig. 6D).

In Table II a pharmacokinetic comparison is made between the three routes of injection for liposomes of the two compositions which resulted in the highest blood levels following sc administration, HSPC/CHOL/PEG-DSPE (2 : 1 : 0.1, molar ratio) and PC40/

TABLE II

Pharmacokinetics in blood of liposomes given by three different routes of administration

Liposomes were extruded MLV, 90 nm in diameter. Mice received 10 μmol/mouse via the tail vein. The calculation for AUC for free ^{125}I-TI assumes that all of the label was released instantaneously from a theoretical liposome dose of 10 μmol/mouse.

Composition	Route of administration	AUC (nmol h/ml)	$T_{1/2\alpha}$	$T_{1/2\beta}$
HSPC/CHOL/PEG-DSPE	iv	355377		18.0
	ip	362098	0.8	17.0
	sc	145453	10.6	19.0
PC40/CHOL/PEG-DSPE	iv	308758		24.3
	ip	251123	0.4	24.7
	sc	141201	7.5	18.2
Free ^{125}I-TI	sc	947		0.125

CHOL/PEG-DSPE (2:1:0.1, molar ratio), and for injection of the free ^{125}I-TI label. The area under the blood level vs. timecurve (AUC) was very similar following iv and ip administration of liposomes of each composition. Following sc administration, the AUC was reduced approximately in half. The $T_{\frac{1}{2}\alpha}$, which provides a measure of the rate of appearance of liposomes in blood after administration by the either the ip or sc routes of injection, was on average approx. 0.6 h following ip administration of liposomes and approx. 9 h following sc administration of liposomes of different compositions (Table II). There was a tendency for liposomes containing saturated phospholipids to be released into blood somewhat more slowly (0.8 and 10.6 h for ip and sc injections, respectively) than liposomes containing unsaturated phospholipids (0.4 and 7.5 h for ip and sc injections, respectively) (Table II). Notably, the $T_{\frac{1}{2}\beta}$, which provides a measure of the rate of removal of liposomes from the blood, was not significantly different for any of the routes of liposome administration, and averaged 20.4 ± 3.4 h (Table II). This, along with the tissue distribution data of Fig. 4 indicates that the liposomes receive equal treatment once they enter the circulation, regardless of the route of administration.

Discussion

Small liposomes (< 120 nm in diameter) containing PEG-DSPE, along with their contents, could move to a surprising extent from a sc site of injection into the bloodstream. It appears that movement occurs down the chain of lymph nodes draining the site of injection and that movement from the site of injection into the lymph nodes may occur through a 'pore' which has a maximum diameter of approx. 110–120 nm. The diameter of this 'pore' appears to be similar to that governing migration of particulate matter from the blood through fenestrated endothelium, e.g., to gain access to

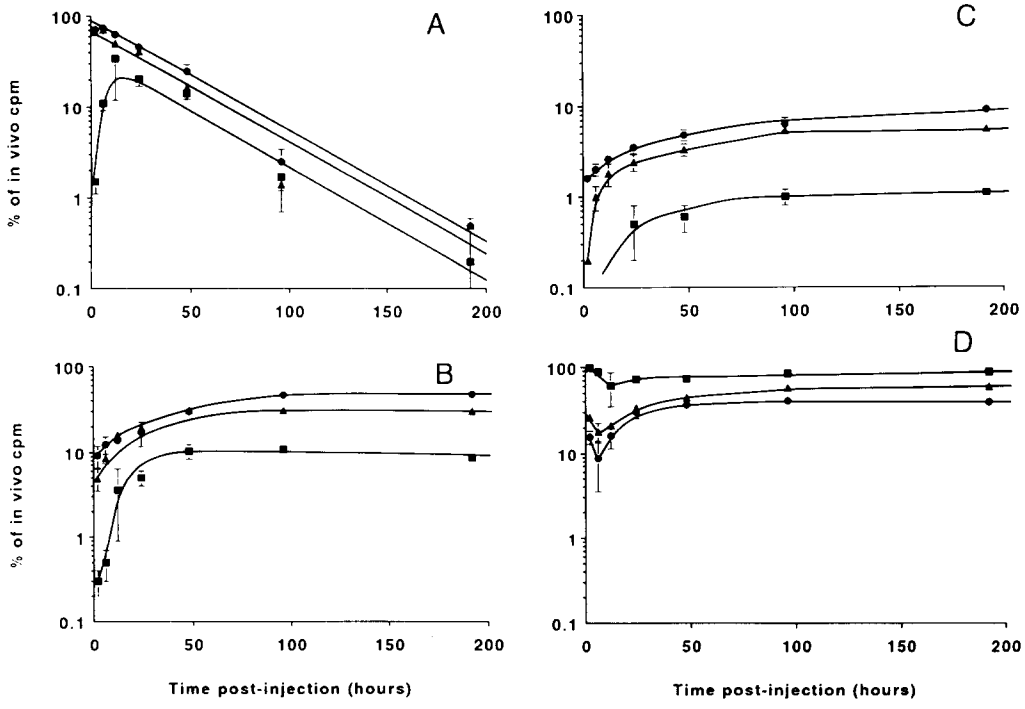

Fig. 6. Tissue distribution of liposomes as a function of iv (●), ip (▲) and sc (■) routes of injection. Liposomes were composed of PC40/CHOL/PEG-DSPE (76 nm, 10 μmol/mouse). Results are expressed as % of in vivo cpm ± S.D. (A) blood, (B) liver, (C) spleen, (D) carcass.

liver parenchymal cells. The 'pore' may simply represent the diameter of the interstial spaces between cells through which liposomes must percolate in order to reach the lymph nodes. Alternative explanations for the size cutoff could include endocytosis of the small liposomes by lymph node macrophages and travel to the blood within these cells, not as free liposomes. However, all liposomes in blood were associated with the plasma fraction, not the cellular fraction. Also, if this were the mechanism, it would be impossible to explain the observation that the pharmacokinetics of small liposomes, once they reach the blood, is identical to free (not cell-associated) liposomes. It is also possible that the small liposomes cross the vascular endothelium by a transcytosis process which might also be size dependent. However, in experiments currently in process in which we are studying the ability of small liposomes to transfer across in vitro models of capillary endothelium we find intact, healthy endothelium almost completely impenetrable to liposomes.

Large liposomes, even those containing PEG-DSPE, are not able to enter the circulation to a significant extent: indeed they do not even leave the site of injection. When liposomes lack added PEG-lipid, even those of small size have considerably lower blood levels, likely because they are taken up by macrophages either at the site of injection, or as they move through the lymph nodes, particularly when low doses are given. High doses, which may saturate the ability of lymph nodes to take up the liposomes, appear to increase blood levels. The much higher uptake of PC40/CHOL/PG liposomes into lymph nodes, at the early time points, suggests that these liposomes are being retained in the lymph nodes to a greater extent, contributing to the much lower blood levels seen for small liposomes of this composition as compared to PEG-liposomes. Furthermore, even when the liposomes lacking PEG-DSPE reach blood, they are rapidly removed into liver and spleen, and blood content of these liposomes never reaches high levels. Ingestion of liposomes at the site of injection might then lead to MPS cells percolating down the lymph node chain with their cargo of liposomes. However, even those small PC40/CHOL/PG liposomes which reached blood were associated with the plasma, and not the cellular fraction of blood, which suggests that MPS cells are not a significant part of the mechanism for transport of liposomes to the blood.

The ability of conventional liposomes to be ingested by bone marrow-derived macrophages in vitro, and the ability of S-liposomes to avoid this fate has been previously described [21]. This reduced recognition of small PEG-liposomes by MPS cells appears to be the key to their ability to achieve high blood levels after sc injection. However, the continued high levels of liposomes in lymph nodes, even at 8 days post-injection (Fig. 5A), suggests that small PEG-liposomes may be retained in lymph nodes to some extent. In vivo, the kinetics of uptake of S-liposomes by MPS cells is much slower than for conventional liposomes and MPS uptake of S-liposomes has been hypothesized to be controlled by the rate at which the PEG is removed from the liposomes resulting in their conversion to conventional liposomes [9].

Less than 1% of injected large liposomes, with or without PEG-lipid, could be found in either liver or spleen, further evidence that they were not able to enter the circulation. We have previously reported that PEG-containing liposomes can reach the blood stream in significant quantities following ip administration [11]. Once they reach the circulation after either sc or ip administration, the liposomes appear to be treated in an identical fashion to liposomes that have been administered by the iv route of injection, a fact which can only be explained by the hypothesis that small S-liposomes reach the circulation intact.

We have interpreted the ^{125}I counts remaining in the body at long time periods post-injection, i.e., 16 days, as providing a possible measure of liposome stability. There are, however, two assumptions associated with this interpretation: the ^{125}I-TI remains in its original form without degradation, and the label resides in liposomes. Degradation of ^{125}I-TI, when it leads to release of free ^{125}I, results in high levels of ^{125}I associated with thyroid. Although thyroid was one of the tissues sampled in all experiments we have not observed high thyroid levels of label (not shown), suggesting that release of free ^{125}I from the degradation of ^{125}I-TI was not a significant problem. Because of the low levels of liposomes and/or label and the difficulties in separating the two at the site of injection, in lymph nodes, etc., it is impossible to determine whether the label was associated with free liposomes or resided within cells at 16 days post-injection, and undoubtedly the data reflect a combination of the two. However, from previous studies on the uptake of conventional and S-liposomes by macrophages [21] it is reasonable to assume that there might be more of a trend towards label being associated with cells in the case of conventional liposomes and towards free liposomes in the case of S-liposomes.

The AUC following sc administration of small, PEG-containing liposomes is surprisingly high, and approaches 50% of the AUC found after iv or ip administration of liposomes of the same composition, size and dose. The AUC for free ^{125}I-TI, if we assumed for the sake of argument that the label, instead of being injected as free label, was instantaneously released from liposomes upon sc injection, was calculated at 47.3 and 947 nmol h/ml for theoretical doses of 0.5 and 10 μmol PL/mouse, respectively. This is only a tiny fraction of the AUC found after injection of liposome-en-

trapped label, and provides a further argument in favour of the label representing intact liposomes. The AUC after sc administration of PEG-containing liposomes of approx. 140000 nmol h/ml, at a dose of 10 μmol PL/mouse, can be compared to AUCs of 924 and 89295 nmol h/ml for iv administration of PC/CHOL, 2:1 liposomes (110 nm diameter) at doses of 0.5 and 10 μmol/mouse, respectively [9]. In other words, sc administration of small S-liposomes (which have dose-independent pharmacokinetics [9]) resulted in higher AUC than administration of MPS-saturating doses of small conventional liposomes.

An application suggested by the high AUC for the liposomal carrier following sc administration is in the area of depot, sustained drug release, in particular for drugs which are rapidly metabolized when given as free drug. Experiments on the therapeutic efficacy of cytosine arabinoside entrapped in S-liposomes, as compared to conventional liposomes, in the treatment of murine L1210 leukaemia has shown that equivalent increases in mean survival times could be obtained for S-liposomes given by the either the sc or iv routes of injection. However, in the case of conventional liposomes the sc route of injection was significantly less efficacious than the iv route [22]. The ability of sc-administered S-liposomes to readily access lymph nodes suggests that there may be applications for antibody-mediated targeting of liposome-entrapped therapeutic drugs to diseased lymph nodes, for example in the case of metastatic cancer.

It has previously been shown that PEG-containing liposomes have significant advantages over conventional liposomes in their long circulation half-lives and dose-independent pharmacokinetics [9]. The ability of sc-administered S-liposomes to move in considerable quantities into the circulation, our ability to manipulate the rate and extent of release into circulation and the liposome stability over a wide range, the high bioavailability of drugs entrapped in these liposomes, along with further reductions in uptake into MPS tissues provide further advantages for PEG-containing liposomes over conventional liposomes.

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