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Sterically stabilized liposomes: Improvements in pharmacokinetics and antitumor therapeutic efficacy

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ABSTRACT **The results obtained in this study establish that liposome formulations incorporating a synthetic polyethylene glycol-derivatized phospholipid have a pronounced effect on liposome tissue distribution and can produce a large increase in the pharmacological efficacy of encapsulated antitumor drugs. This effect is substantially greater than that observed previously with conventional liposomes and is associated with a more than 5-fold prolongation of liposome circulation time in blood, a marked decrease in uptake by tissues such as liver and spleen, and a corresponding increased accumulation in implanted tumors. These and other properties described here have expanded considerably the prospects of liposomes as an effective carrier system for a variety of pharmacologically active macromolecules.**

Over the last 20 years, liposomes have served a dual role, as a valuable experimental tool for membrane research and in addition as an *in vivo* delivery system for enhancing the efficacy of various pharmacologically active molecules (1). Animal studies have shown that liposomes can decrease the toxicity of several antitumor and antifungal drugs, leading to several recent clinical trials with promising results (2–6). In addition, liposomes have been shown to be efficient carriers of antiparasitic drugs for treating intracellular infections of the reticuloendothelial system (RES), in activating macrophage cells to become tumoricidal in models of metastasis, and in enhancing the immune response to encapsulated antigens, thus facilitating the formulation of artificial vaccines (7, 8). All these effects stem from the capacity of macrophage cells in the liver and spleen (mononuclear phagocytic system or RES) to remove the majority of liposomes from the blood circulation within minutes (9). Such rapid clearance of conventional liposomes [i.e., liposomes composed of various phospholipids and cholesterol (Chol) and possibly other lipids, without specific components conferring the property of long circulation in blood] from the circulation, however, has limited their prospects as an *in vivo* delivery system for transporting drugs to sites of disease beyond the RES.

Recent reports from our laboratories have described liposome formulations (containing the monosialoganglioside G_{M1} or phosphatidylinositol) that exhibit a prolonged circulation time in blood and diminished uptake by the liver and spleen (10–12) and increased accumulation in implanted tumors (12–14). The term "stealth" has been proposed (15) for these long-circulating liposomes. We consider that such liposomes are sterically stabilized by the presence of the relevant headgroups on their surface (16). In this report we present several findings obtained with liposomes sterically stabilized by the PEG headgroups of a synthetic phospholipid (PEG-DSPE) included in the formulation (PEG-liposomes). Preliminary experiments with PEG-liposomes have been published recently (17–19). New microscopic evidence indicates that accumulation of liposomes in tumors involves extravasation, presumably due to increased permeability of the capillary endothelia (20, 21). Unlike conventional liposomes, which show dose-dependent blood clearance kinetics (9, 22, 23), the PEG-liposome formulations show prolonged circulation time in blood, with clearance kinetics that are completely independent of dosage over a wide range. Most importantly, they produce a marked enhancement of the antitumor activity of encapsulated doxorubicin and epirubicin in mice against both i.p. lymphoma and s.c. colon carcinoma, with a concomitant decrease in toxicity. This indicates an increase in the therapeutic index to a value that is much higher than that observed with conventional liposome formulations.

MATERIALS AND METHODS

Materials. Egg phosphatidylcholine (EPC), distearoyl phosphatidylcholine (DSPC), phosphatidylglycerol (PG) derived from EPC (EPG), and bovine brain sphingomyelin (SM) were purchased from Avanti Biochemicals (Birmingham, AL). The monosialoganglioside G_{M1} was obtained from Makor Chemical (Jerusalem). Chol was from either Sigma or Croda (Fullerton, CA). *N*-Tris[hydroxymethyl]-2-aminoethanesulfonic acid (Tes) was purchased from Sigma. Hydrogenated soy phosphatidylcholine [HSPC; NC-100-H, containing 98.2% phosphatidylcholine (PC) and 1.8% lyso-PC] was purchased from Natterman (Cologne, F.R.G.). Partially hydrogenated egg phosphatidylcholine (PHEPC) 99% pure, with an iodine value of 40, was obtained from Asahi Chemical Industries (Tokyo). Tyraminylinulin was synthesized and ^{125}I-labeled tyraminylinulin was prepared as before (24). Na^{25}I was obtained from the Edmonton Radiopharmaceutical Center; pyrogen-free saline for injection (0.9%, USP) was from Travenol Canada (Mississauga, ON). All test tubes, syringes, etc. were sterile and pyrogen-free. Desferal–gallium-67 (^{67}Ga-DF) was prepared as described (25). Dextran labeled with rhodamine B isothiocyanate (Rh-Dex; $M_r \approx$ 9000) was purchased from Sigma. Doxorubicin and epirubicin were obtained from Farmitalia, Carlo Erba (Milan). PEG (M_r = 1900)-derivatized distearoyl phosphatidylethanolamine (PEG-DSPE) was synthesized as described (26).

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Abbreviations: RES, reticuloendothelial system; EPC, egg phosphatidylcholine; EPG, phosphatidylglycerol derived from EPC; SM, sphingomyelin; HSPC, hydrogenated soy phosphatidylcholine; PEG-DSPE, polyethylene glycol conjugated to distearoyl phosphatidylethanolamine; ^{67}Ga-DF, Desferal–gallium-67; Rh-Dex, dextran labeled with rhodamine B isothiocyanate; Chol, cholesterol; PC, phosphatidylcholine; PG, phosphatidylglycerol; DSPC, distearoyl phosphatidylcholine; PHEPC, partially hydrogenated EPC.

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Liposome Preparation. Liposomes were prepared by thin film hydration as described (10, 12). The resulting multilamellar vesicles at lipid concentrations of ≈10 μmol/ml were frozen (at −40°C) and thawed (at 60°C) several times and then passed repeatedly under pressure through 0.2- or 0.1- and 0.05-μm pore size polycarbonate membranes (27), and the extrusion was repeated until the average size distribution was ≈80–100 nm in diameter (mean Gaussian distribution by volume). Liposome morphology was occasionally examined by electron microscopy (28, 29).

For encapsulation of ^{67}Ga-DF, the liposomes were prepared with Desferal, and the ^{67}Ga was loaded just before use (25). Iodinated tyraminylinulin was encapsulated as before (10). Rh-Dex (M_r = 9000) was encapsulated passively by inclusion of the material at a concentration of 100 mg/ml in the preparation buffer. Free Rh-Dex was removed by gel permeation on a Sephadex G-150 column and an Amicon concentration unit with a M_r 30,000 cut-off filter. Liposomes containing doxorubicin or epirubicin were composed of PEG-DSPE/HSPC/Chol/α-tocopherol (0.15:1.85:1.00:0.018, mol/mol) and were prepared as described (30). The drug and total lipid concentrations of the final product were about 2 mg/ml and 20 μmol/ml, respectively (±10% for both), and the particle diameter was 85 ± 10 nm.

Animal Experimentation. Experiments with labeled inulin were performed in female ICR (outbred) mice (10), and tissue distribution experiments were performed with ^{67}Ga-DF in normal Swiss–Webster mice (12) and in tumor-bearing BALB/c mice inoculated s.c. with the C-26 colon carcinoma (31). Mice (three per group) were injected in the tail vein with ≈0.2 ml of 0.1–10 μmol of phospholipid. Blood correction factors (10, 12) were applied to tissues and the carcass. Male and female adult Sprague–Dawley rats (220–400 g) were surgically prepared under anesthesia for i.v. administration of a 0.3- to 0.4-ml sample via a femoral venous cannula and blood sampling via chronic femoral arterial cannula externalized at the back of the neck. The animals were awake and unrestrained during blood drawing except for the initial two time points, 1 and 15 min. The amount of lipid injected was 10–15 μmol of phospholipid per kg of animal weight.

Mouse colon carcinoma (C-26) cells were injected into BALB/c female mice (6–9 weeks old) as before (31). At day 0, mice were injected into the left flank s.c. with 4×10^5 cells in a volume of 0.1 ml. One day later drug was injected i.v. (tail vein, 0.2 ml or less, mouse weight between 16 and 21 g). Treatment was repeated at weekly intervals to a maximum of three injections. Tumor volume was measured as before (31).

The lymphoma cell line J6456 was inoculated i.p. into BALB/c female mice (32). When there was about 2 ml of ascitic fluid accumulated, containing between 10^8 and 10^9 tumor cells, the drug was injected i.v. either free in solution or in encapsulated form. The ascitic fluid was thereafter sampled at different intervals. Tumor cells were separated by centrifugation. Part of the supernatant was passed through a Dowex column to remove the non-liposome-associated drug (33). The drug concentration was determined as before (30). For therapeutic experiments, BALB/c female mice (20 in each treatment group) were inoculated i.p. on day 0 with 10^6 lymphoma cells (J6456). Treatment consisted of a single i.v. injection on day 5 containing either free or liposome-encapsulated doxorubicin.

RESULTS

Tissue Distribution and Pharmacokinetics. Recent studies with PEG-liposomes in mice demonstrate blood levels comparable to G_{M1} formulations (10, 12): 24–30% of total recovered dose at 24 h, regardless of whether the PC was rigid (DSPC) or fluid (EPC). In addition, similarly high liposome recoveries were obtained even when EPG was included in addition to PEG-DSPE. Average values for the percent recovered dose of PEG-DSPE/EPC/Chol liposomes (compared to the conventional formulation of EPG/EPC/Chol) at 24 h following i.v. injection in mice were as follows: blood, 29.3 ± 2.8 (1.2 ± 0.3); liver 17.9 ± 2.0 (61.3 ± 3.4); spleen, 2.7 ± 0.6 (4.3 ± 0.4); heart, 0.1 ± 0.7 (0.3 ± 0.1); skin, 15.6 ± 2.0 (4.7 ± 0.9); carcass, 20.5 ± 2.7 (15.3 ± 3.1). Our definition for carcass excludes all major organs such as liver, spleen, heart, kidneys, gut, and lungs.

We have also determined the blood clearance kinetics of several liposome formulations in rats by repeated sampling of blood, using ^{67}Ga-labeled liposomes as above. Fig. 1 compares the clearance curve of free ^{67}Ga-DF with that of the same label when encapsulated in liposomes of conventional composition (EPG/PHEPC/Chol or PHEPC/Chol) and when encapsulated in PEG-liposomes (PEG-DSPE/PHEPC/Chol). It is noteworthy that the clearance curve of the liposomes of the latter composition is linear on a semilogarithmic plot (Fig. 1), with a 5-fold increased $t_{1/2}$ value (to 15.3 h), as opposed to the presence of a fast and a slow component of clearance obtained with conventional liposomes, which show saturation of one or more of the components of uptake at doses of 40 mg/kg or lower (34). Pharmacokinetic studies with PEG-liposomes (Figs. 1 and 2) and additional work elsewhere (35) indicate that these compositions show single first-order clearance rates that are independent of dose over the entire dosage range examined (0.5–500 μmol/kg in mice and 3–70 μmol/kg in rats). Fig. 2 compares the blood levels and RES uptake obtained with PEG-DSPE/EPC/SM/Chol and EPC/Chol liposomes at 2 h after i.v. injection. These results indicate high, dose-independent blood levels for PEG-liposomes (35) as well as independence for the amount of label accumulating in the liver and spleen (Fig. 2*A*). Conventional liposomes (EPC/Chol) show decreasing percentages accumulating in liver and spleen with increasing dose, reaching a plateau at doses of >2 μmol of phospholipid per mouse. The results described above suggest that PEG-liposomes are not being recognized by high-affinity, saturable, binding sites, such as those expressed on resident macrophages in the liver and spleen, which recognize conventional liposomes (36). Indeed, the uptake of sterically stabilized liposomes *in vitro* by bone marrow-derived murine macrophages (37) and by CV1 cells, a primate cell line of epithelial origin (38), is significantly reduced compared to conventional liposomes.

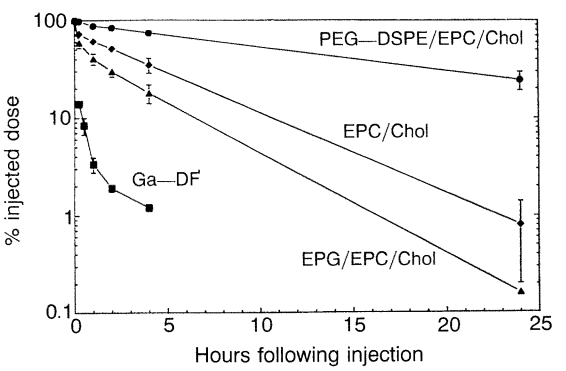

FIG. 1. Blood clearance kinetics of liposomes in rats. ^{67}Ga-DF was used as a marker for liposome contents. Results are expressed as the percentage of the injected dose present in blood at any particular time. Error bars represent the average (± SD) of three different experiments. ■, free ^{67}Ga-DF; ▲, ^{67}Ga-DF encapsulated in EPG/EPC/Chol (0.15:1.85:1, mol/mol) liposomes; ◆, ^{67}Ga-DF encapsulated in EPC/Chol (2:1, mol/mol) liposomes; ●, ^{67}Ga-DF encapsulated in PEG-DSPE/EPC/Chol (0.15:1.85:1, mol/mol) liposomes. In this figure, EPC stands for PHEPC.

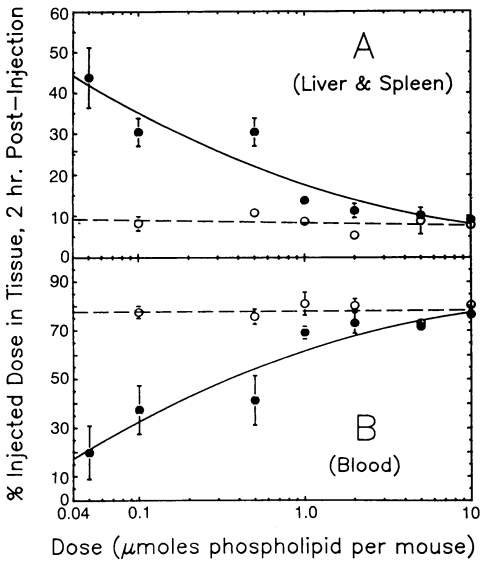

FIG. 2. Dose dependence of liposome distribution in mice. ^{125}I-labeled tyraminylinulin was used as a marker of liposome contents. The results are plotted as the percentage of the injected dose present 2 hours after injection in liver and spleen (corrected for blood) (*A*) or in blood (*B*). Each point represents an average (± SD) from three animals. One micromole per mouse represents a dose of 50 μmol/kg. ●, EPC/Chol (1:1, mol/mol) liposomes; ○, PEG-DSPE/EPC/SM/Chol (0.14:1:1:1, mol/mol) liposomes.

Uptake by Tumors and Extravasation of Liposome Label and Drug. *Accumulation in s.c.-implanted colon carcinoma.* We have compared liposomes composed of DSPC/Chol and PEG-DSPE/DSPC/Chol at the same dosage and particle size. The amount of ^{67}Ga present at different times in blood, liver, and tumor (C-26 cells injected s.c. 2–3 weeks earlier) was expressed as the percentage of the injected dose per gram of tissue. It was found that inclusion of PEG-DSPE increases the amount of label found in blood (by 4-fold) and tumor (by 2.3-fold) and decreases the amount found in liver (by 2-fold) at 48 h after i.v. injection. It should be noted here that DSPC/Chol and also SM/Chol have the longest circulation times compared to other conventional compositions (39–41). However, unlike sterically stabilized liposomes, they still show dose-dependent kinetics and saturation of liver uptake at high concentration (39). When a negatively charged phospholipid such as PG or phosphatidylserine was added to either of these two compositions, the uptake by the liver was much higher (12, 42) and that of the tumor much lower (12, 14).

We have also examined the tumor tissue microscopically to determine whether the accumulated liposomes have extravasated beyond the endothelial barrier of the tumor vasculature. Rh-Dex was used as a water-soluble liposome-encapsulated marker to study the distribution of liposomes within the tumor mass by fluorescence microscopy. Fig. 3 shows a phase-contrast picture of a frozen section of tumor tissue, along with a picture of the same section observed in dark-field fluorescence microscopy. When a solution of non-encapsulated Rh-Dex was injected i.v., there was no detectable fluorescence in the tumor after 24 h. The photograph shown in Fig. 3 is representative of a large number of photographs taken from various sections of the tumor mass. An uneven focal distribution of the fluorescence was observed within the tumor, with both dark areas and other highly fluorescent areas. Similar localization was observed by using a lipid fluorescent marker, rhodamine-labeled phosphatidylethanolamine, indicating that the fluorescence revealed by Rh-Dex in Fig. 3 probably represents intact liposomes. The fluorescence was localized well beyond the endothelial layer and was apparently extracellular, in the interstitial space surrounding living tumor cells. This conclusion has been confirmed more recently by silver enhancement of gold particles encapsulated into liposomes (43).

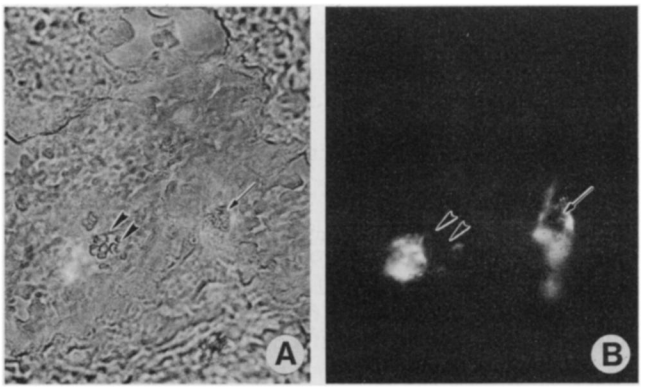

FIG. 3. Localization of liposomes in C-26 colon carcinoma. Liposomes with encapsulated Rh-Dex were injected into the tail vein of mice implanted with s.c. tumor. Colon carcinoma cells (C-26) were inoculated s.c. into BALB/c mice 2–3 weeks before the liposomes. Tumors at that time weighed ≈1 g. At 24 h after liposome injection, mice were perfused with heparinized buffer and subsequently with 4% paraformaldehyde. The tumors were collected and further fixed in 1% glutaraldehyde. Frozen sections of the tumors (≈5 μm thick) were examined in a fluorescence microscope with ×400 magnification. Most of the fluorescence signal was grossly associated with the vasculature inside the tumor mass, whereas a gradient of fluorescence away from them was usually observed. (*A*) Phase-contrast image of a representative section. (*B*) Corresponding fluorescence micrograph. Arrows and arrowheads point to an area with red blood cells in the blood vessel, which is dark in *B*. Rhodamine fluorescence is bright surrounding and away from that area, well beyond the endothelial layer of the vasculature.

Accumulation in i.p.-implanted lymphoma. The experimental model consisted of lymphoma grown i.p. with an i.v. injection of PEG-DSPE/HSPC/Chol liposomes encapsulating doxorubicin, an anthracycline used widely as an antitumor agent. Fig. 4 shows the concentration of drug in the liver,

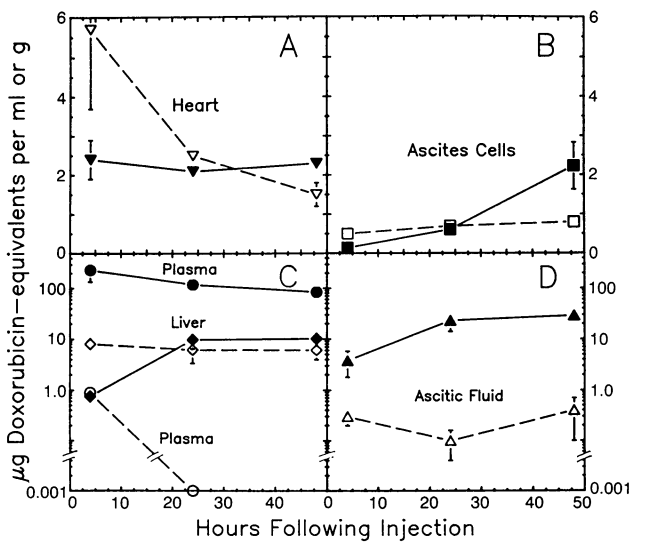

FIG. 4. Liposome-encapsulated doxorubicin in tumor-bearing mice. The presence of doxorubicin was measured directly by fluorimetric determination in various tissues of BALB/c mice inoculated (i.p.) with J6456 lymphoma, at different times following injection either as free drug (open symbols, dashed lines) or encapsulated in liposomes (closed symbols, solid lines) composed of PEG-DSPE/HSPC/Chol (0.2:2:1 mol/mol). ◇ and ◆, liver; ▽ and ▼, heart; ○ and ●, plasma; △ and ▲, ascitic fluid; □ and ■, ascitic cells.

heart, plasma, and ascitic fluid after i.v. injection of doxorubicin either free or encapsulated in PEG-liposomes. The ascitic fluid is an extravascular/extracellular fluid pool and is in direct contact with the tumor cells. The results indicate that encapsulation in PEG-liposomes diminished the early uptake of the drug by the heart and liver (lower values at 4 h) although the uptake in the liver was increased after 24 h, as observed earlier (12, 14). Most importantly, there was a much higher concentration of the drug in plasma and in the ascitic fluid with the encapsulated formulation compared to the free drug. Separation of free drug on a Dowex column (30) showed that the majority of the drug present in the ascitic fluid was in intact liposomes and only 15–25% was free drug between 24 and 48 h. The concentration of the drug in the ascitic cells (mostly tumor cells) was initially lower but increased with time (Fig. 4). These results, taken altogether, indicate that PEG-liposomes initially injected i.v. can extravasate into the peritoneal cavity where they provide a constantly high reservoir of encapsulated drug, available to be taken up by the tumor cells. Animals carrying no tumors did not accumulate any appreciable amount of liposomes into their peritoneal cavity, although liposomes are known to get transported from the peritoneal cavity to blood (11, 15).

Anti-Tumor Efficacy of Drug-Containing Liposomes. *Mouse colon carcinoma grown s.c.* For the therapeutic experiments, multiple injections of liposomes composed of PEG-DSPE/HSPC/Chol loaded with epirubicin were given i.v. starting 1 day after s.c. inoculation of 4×10^5 C-26 tumor cells. The dosage of drug per injection was 6 and 12 mg/kg, and the initial injection was repeated twice (total of three injections) at 7-day intervals. The antitumor effects were registered by measuring tumor size and the survival of mice up to 120 days after tumor implantation. The results presented in Fig. 5 show clearly that encapsulation causes a marked improvement in therapeutic efficacy, inhibiting the tumor growth (Fig. 5*A*) and producing a large percentage of

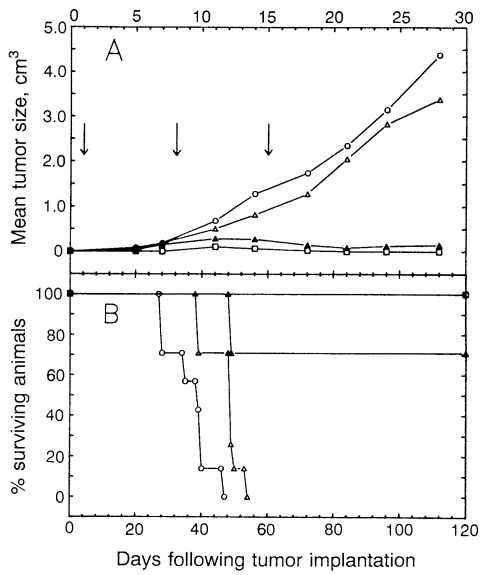

FIG. 5. Therapeutic experiment with colon carcinoma in mice. Colon carcinoma cells (C-26) were inoculated s.c. into BALB/c mice. Treatment started on day 1 and was repeated twice on days 8 and 15; it consisted of an i.v. injection of epirubicin either free or encapsulated in liposomes composed of PEG-DSPE/HSPC/Chol. There were four treatments as follows: ○, saline control; △, free epirubicin at 6 mg/kg (nonencapsulated); ▲, encapsulated epirubicin at 6 mg/kg; □, encapsulated epirubicin at 12 mg/kg. (*A*) Measurement of mean tumor size (volume in cm^3) between 0 and 30 days after tumor implantation. (*B*) Survival curves expressed as the percentage of surviving animals from 0 to 120 days postimplantation. The total number of animals was 6–10 per group.

long-term survivors (Fig. 5*B*). The controls with injections of free drug or drug and empty liposomes showed only a slight prolongation of mean survival over untreated animals. Similar effects were obtained when liposomes were injected with a delay of 3 or 10 days, and the effect was smaller but still significant, even with a delay of 14 days (E.M., D.D.L., S. Babar, and F. J. Martin, unpublished work).

The doses of free and encapsulated drug were chosen on the basis of toxicity experiments performed in animals without tumors, with a similar regime of three doses at weekly intervals. These studies indicated that a dose of epirubicin at 9 mg/kg encapsulated in liposomes and at 6 mg/kg as free drug resulted in no deaths up to 120 days following injection. In the same period three deaths out of five animals were observed with epirubicin at 12 mg/kg encapsulated in liposome and at 9 mg/kg as free drug. On the basis of these results, we can conclude that the acute toxicity of epirubicin, as exemplified by the maximum tolerated dose, is reduced slightly following liposome encapsulation. As already shown in Fig. 5, the therapeutic effect for a similar dose was increased markedly by liposome encapsulation. Thus, the therapeutic index of this drug (ratio of efficacy to toxicity) is considerably increased by encapsulation in PEG-liposomes. Earlier work had indicated that the C-26 colon carcinoma grown s.c. is not responsive to doxorubicin encapsulated into liposomes of conventional formulations (31).

Mouse lymphoma grown i.p. The antitumor efficacy of liposome-encapsulated doxorubicin was tested in this tumor model system after i.v. injection. The median survival time of the control (untreated) mice was 16 days. It was increased to 24 and 21 days with a single dose of free doxorubicin at 10 and 15 mg/kg, respectively. Encapsulation in PEG-DSPE/HSPC/Chol liposomes resulted in a substantial lengthening of the median survival to 30 and 44 days, respectively, for doses of 10 and 15 mg/kg. The last dose also resulted in two long-term survivors. Toxicity experiments with increasing doses (15, 20, and 25 mg/kg) of doxorubicin encapsulated in PEG-liposomes as above showed a substantial decrease in acute toxicity as compared to the free drug. From a comparison of the efficacy and the toxicity results, we conclude that encapsulation in PEG-liposomes produces a significant increase in the therapeutic index of doxorubicin against mouse lymphoma.

DISCUSSION

The clearance rate of liposomes from blood (41, 44, 45) as well as their accumulation in tumors (14) depends on particle size in addition to specific lipids (10, 12). Accumulation of small (≈50-nm diameter) neutral liposomes (DSPC/Chol) in tumors had been observed earlier (46). In our studies (12, 14) we have enhanced this accumulation by introducing liposome formulations with longer blood half-life and have increased their usefulness as a drug carrier by using liposomes of ≈100-nm diameter, which have a 16-fold higher internal volume as compared with smaller (50-nm) liposomes. We have recently proposed a mechanism for the prolonged blood clearance involving steric stabilization of the liposome surface (16).

The high recovery in blood reported here with PEG-liposomes and their accumulation in tumors (Fig. 3) may prove to be of considerable advantage to future clinical applications for the following reasons. (*i*) The PEG derivative is a synthetic phospholipid easily prepared at high purity in large quantities and considerably lower cost compared to the G_{M1} ganglioside, which is derived from bovine brain. (*ii*) Fluid and solid bilayer compositions give similar blood circulation times, adding considerably to the flexibility needed for various clinically relevant formulations. (*iii*) The additional presence of the negatively charged PG in the liposome

bilayer allows for better encapsulation of a variety of positively charged drugs and other macromolecules and reduces the likelihood of aggregation. (*iv*) Dose independence dictates that a constant percentage of the injected dose is taken up by various tissues, irrespective of the amount injected, allowing for a reliable prediction of drug levels during therapy (35). Finally, (*v*) reduced uptake by liver and spleen decreases the possibility for RES toxicity and enhances the chances for localization in other tissues for therapeutic and imaging applications.

Our therapeutic studies with PEG-DSPE/HSPC/Chol liposomes showed a significant increase in the therapeutic index of antitumor drugs in mice against both a lymphoma and a colon carcinoma. This is consistent with our earlier findings that phosphatidylinositol liposomes have a pronounced effect on the blood clearance kinetics of doxorubicin (30) and the accumulation of liposome markers in implanted tumors (12–14). The lower acute toxicity of anthracycline-loaded PEG-liposomes reported here is similar to that observed earlier with conventional liposomes (47–50). It is probably related to the delayed clearance of drug from the blood and the reduction of peak plasma levels of free drug, which adversely affect sensitive tissues such as the heart and others involved in the acute toxic effects. Whether the increased therapeutic efficacy is due to the improved (delayed) blood clearance as seen recently with arabinose cytosine encapsulated in G_{M1}-liposomes (51) or the increased local accumulation of drug within the tumor mass is not certain at present. It is possible that both factors contribute, although the latter is a more likely possibility.

The microscopic studies discussed above have indicated the presence of PEG-liposomes in the interstitial space around tumor cells. It is quite possible that their extravasation beyond the endothelial layer in tumors is the result of their long circulation time in blood and the increased permeability of the endothelial barrier in the newly vascularized tumors (12, 20, 21). Although diffusion and deep penetration within the tumor mass is not expected for particles of the size of liposomes (20, 21), it is possible that free drug can diffuse locally from the extravasated liposomes to the surrounding tumor cells. The presence of intact extracellular liposomes within the tumor area would provide a local depot for drug release. Enhanced localization of liposomes in other anatomically distinct regions might also be beneficial for other diseases involving areas of increased capillary permeability.

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