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Liposome leakage and blood circulation: Comparison of adsorbed block copolymers with covalent attachment of PEG

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Summary

Adsorption of block copolymers onto colloids is a promising method to reduce their biological interactions leading to phagocytosis that, for liposomes in particular, is challenged by recent competition through covalent coating with polyethyleneglycol (PEG). The interaction of block copolymer emulsifying agents and PEG lipid derivatives with liposomes was investigated for evidence of adsorption though dynamic light scattering measurements of mean particle size distribution and compared with those of entrapped aqueous label efflux. The results show increases in hydrodynamic radius are less than about 3.5 nm, regardless of liposome composition or preparation method. At the same time, efflux of entrapped aqueous label increases indicating that adsorption does occur. Efflux was reduced but not eliminated when the liposomes were prepared with high temperature phase transition lipids. In contrast, efflux was not observed upon addition of the PEG lipid derivative. In vivo studies of liposomes treated with the block copolymer, F-108, showed a moderate increase in blood circulation time, but less than that when the PEG lipid derivative was incorporated.

Introduction

Extensive investigations in many laboratories have shown that liposomes possess great promise as a colloidal drug delivery system with several advantages. They have low toxicity, are only weakly immunogenic, and are biodegradable. The many technical requirements for their pharmaceutical application have been addressed by extensive efforts by many workers (for recent reviews see Fidler, 1988; Gregoriadias, 1988; Riaz et al., 1988). However, despite the similarity of liposomes with a variety of native biological structures they are rapidly cleared from blood by cells of the mononuclear phagocytic system (MPS), originally referred to as the reticuloendothelial system (RES). Many approaches to decrease interaction with biological components and thereby increase the blood circulation have been reported such as coating with proteins, polysaccharides, or glycolipids (Torchillin et al., 1980; Sunamoto and Iwamoto, 1986; Allen and Chonn, 1987; Gabizon

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and Papahadjopoulos, 1988). One approach, based on methods described for coating latex colloidal particles with amphipathic polymers (Illum and Davis, 1984; Illum et al., 1987), has been applied to lipid stabilized emulsions (Davis and Hansrani, 1985; Illum et al., 1989) and liposomes (Senior, 1987; Jamshaid et al., 1988; Moghimi et al., 1991). The results indicated that these polymers can adsorb onto liposomes as found with latex giving an increase in blood circulation. In some cases, though, the adsorption led to decreased retention of aqueous entrapped components (Jamshaid et al., 1988). Another approach reported recently giving prolonged circulation is based on incorporating a polyethylene glycol derivatized lipid (Blume and Cevc, 1990; Klibanov et al., 1990; Woodle et al., 1990, 1992a; Papahadjopoulos et al., 1991; Senior et al., 1991). While the PEG lipid derivatives used in some cases have been shown to be water soluble (Lasic et al., 1991; Woodle et al., 1991), their effect on leakage has not been reported.

In this work, comparison of three block copolymers with a PEG lipid derivative was undertaken to examine their effects on leakage and the relative nature of the prolonged circulation which results. The results show that the block copolymers induce leakage of liposomes, even those prepared with high temperature phase transition lipids, while the PEG-DSPE did not. A moderate increase in blood circulation was achieved when F-108 was added, but which is less than that obtained when PEG-DSPE is incorporated.

Materials and Methods

Liposome preparation

Multilamellar vesicles (MLV) were prepared and either extruded or homogenized by standard methods (Olson et al., 1979; Woodle and Papahadjopoulos, 1988; Woodle et al., 1992a). Briefly, lipid films were formed by rotoevaporation of lipid mixtures in chloroform followed by high vacuum to remove residual solvent. Lipid mixtures were prepared with the following lipids: phosphatidylglycerol (PG) (Avanti Polar Lipids), hydrogenated soy phosphatidylcholine (HSPC) (Avanti Polar Lipids), partially hydrogenated egg phosphatidylcholine with an iodine value of 40 described previously (Lang et al., 1990) (PHEPC, Asahi Chemical Japan, Tokyo Japan), and USP grade cholesterol (Chol) (Croda, Fullerton, CA). The films were hydrated by shaking with an aqueous solution of desferoxamine mesylate (DF, Sigma Chemical, St. Louis, MO) in 0.9% saline for injection above the phase transition temperature of the phospholipid component for 60 min. For extrusion, liposome dispersions containing 10 μM phospholipid/ml were freeze-thawed three times and extruded under high pressure in a stainless-steel cell (MICO, Middleton, WI) through 0.05 μm diameter defined pore filters (Nuclepore) typically until the mean particle diameter was less than or equal to 100 nm. For homogenization, liposome dispersions containing 50 μM phospholipid/ml were homogenized (Minilab, Rannie, Denmark) until the desired mean particle size was obtained. The mean diameter measurements of particle size distribution were determined by dynamic light scattering using a Gaussian distribution (NICOMP Instruments model 200 adapted with a Brookhaven Instruments BI-2030AT autocorrelator). Phospholipid concentrations were measured by phosphorus determination (Bartlett, 1959).

Liposome labeling

Entrapped ^{67}Ga chelated by DF was prepared by modification of a previously reported method (Gabizon et al., 1989–90; Woodle, 1992). Briefly, ^{67}Ga-citrate for injection (Neoscan, NEN Cambridge, MA) was converted to a bilayer permeable oxime chelate (hydroxyquinoline) by diluting the Ga-citrate stock 1:10 to give a solution of 5 mg/ml hydroxyquinoline sulfate (Sigma Chemical Co.) in 0.9% saline, heated to 50°C for 1 h, and added to liposome samples which had been treated to remove any unentrapped DF by gel permeation chromatography on 10DG Econo Pak columns (BioRad). Loading with 0.1–3 μCi/μM lipid gave good results. The samples were capped, mixed, and incubated at 4°C. Unentrapped Ga label was removed by gel chromatography as above.

Determination of leakage

Measurement of the amount of unentrapped label was used to assess leakage of aqueous contents. The percent entrapped label was determined by gravity flow gel permeation chromatography with Sephadex G-50 in a 7 mm × 26 mm column equilibrated with saline using 20 μl loading samples and collection of 0.5 ml fractions.

Adsorption to liposomes

^{67}Ga-DF loaded liposomes were mixed with dilutions of aqueous polymer solutions and allowed to stand for 24 h. These samples were then assayed for particle size and % entrapped label. The results are expressed relative to a control sample mixed with water. The following materials were tested for interaction with liposomes: methoxypolyethylene glycol carbamate of distearylphosphatidylethanolamine (PEG-DSPE) described elsewhere (Woodle et al., 1992a) and polymers polyoxamine 908 (Tetronics 908 or P-908), Pluronic F-127, and Pluronic F-108 (BASF).

In vivo

In vivo studies were performed with male and female adult Sprague Dawley rats (220–400 g) as described previously (Woodle et al., 1992a). Briefly, the animals were surgically prepared for intravenous administration and arterial blood sampling. A 300–400 μl sample was administered via a femoral venous cannula, which was then removed and whole blood samples obtained via a chronically implanted femoral arterial cannula. The blood samples were distributed in triplicate for measurements of ^{67}Ga using a gamma counter (Beckman model 5000). Tissues were removed surgically after the final blood sample was obtained and the levels of ^{67}Ga radioactivity determined with the gamma counter.

Results

Adsorption of block copolymers to liposomes

The interaction with and adsorption of block copolymers to liposomes were investigated by measurements of particle size based on unimodal distributions from dynamic light scattering and leakage of the aqueous contents.The interactions of three block copolymers, P-908, F-127, and F-108, were examined as representatives from two classes: polyoxamine (P-908) and polyoxamers (F-127 and F-108). The P-908 material consists of four polyoxyethylene (POE) polymers of about 5 kDa each attached to a polyoxypropylene (POP) polymer of about 1.25 kDa and all four are coupled to an ethylene diamine giving a total molecular mass of 25 kDa as described previously (Illum et al., 1989). The F-127 and F-108 consist of two polyoxyethylene polymers attached to the ends of

TABLE 1

Changes in mean diameter and % encapsulated ^{67}Ga-desferal from PG PC Chol liposomes after addition of aqueous polymers

Added polymer		Mean diameter (nm)				% entrapped			
wt% [a]	PEG-DSPE (mol%) [b]	PEG-DSPE	Poly 908	F-127	F-108	PEG-DSPE	Poly 908	F-127	F-108
(9 : 1 mixture of liposome sample with aqueous polymer solution)									
0	0	95.0 ± 0.8	95.0 ± 0 8	95.0 ± 0.8	117 ± 1 0	93 6	93.6	93.6	98.0
10					119 ± 1.3				86 5
30	7 5	95.0 ± 0.5			118 ± 1.3	92 3			82.5
60					119 ± 1.0				71.9
100	20	94 8 ± 0 6	95 4 ± 0 5	97.2 ± 0 9		92 0	82 7	43.4	
(1 : 1 mixture of liposome sample with aqueous polymer solution)									
0			95.2 ± 0 7	95.2 ± 0.7			75 4	75.4	
1 000			99 7 ± 1	102.1 ± 1			59 0	39 9	

[a] Amount used for all materials tested.

[b] Calculated from molecular mass for the weight % used.

a polyoxypropylene polymer with molecular masses of 12.6 and 14.8 kDa, respectively. These two materials contain POE portions of molecular mass 9 and 12 kDa, respectively, for F-127 and F-108. Their selection for these studies was made on the basis of previous studies of block copolymers with emulsions (Illum et al., 1989) and liposomes (Jamshaid et al., 1988). As before, the polymers were allowed to interact for 24 h at room temperature before analyses (Jamshaid et al., 1988). Studies with negatively charged liposomes composed of low temperature phase transition phospholipids are reported in Table 1. Up to 100% weight of the polymers per weight of liposome lipids did not result in a change in the liposome mean diameter relative to a control addition without polymer. This is in comparison with increases of 20–30 nm in mean diameter reported with latex (Illum et al., 1986) and EPC SUVs (Jamshaid et al., 1988) resulting from a coating thickness on the order of 10–15 nm. Measurements at even higher weight percent polymer performed by 1:1 dilution of the liposomes with the polymer solution are also listed in Table 1. These results show little increase in mean diameter despite considerable leakage of the contents as determined by loss in % entrapped label. Fig. 1 demonstrates the chromatograms of the liposomes before and after addition of the F-108 polymer. The only size change observed, an increase of about 7 nm in mean diameter at 1000% F-127, represents a coating thickness of about 3.5 nm, much less than

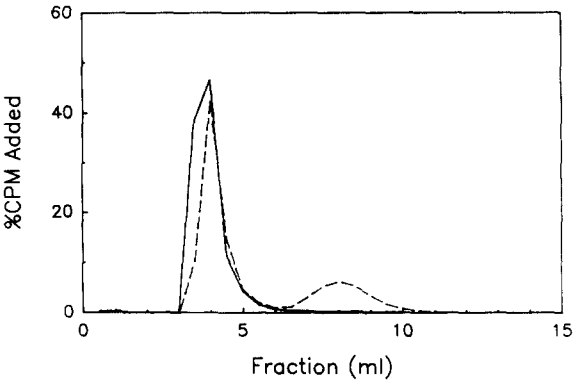

Fig. 1. Gel permeation chromatography profile of ^{67}Ga-labeled PG:PC·Chol liposome with and without block copolymer F-108 added at 60 wt% (data from Table 1) Solid line, without F-108; dashed line, with F-108

observed with latex. Identical liposomes with a mean diameter of about 60 nm gave similar results (data not shown).

Included in Table 1 are results obtained with PEG-DSPE. Liposomes containing this lipid have prolonged circulation in vivo (Blume and Cevc, 1990; Klibanov et al., 1990; Woodle et al., 1990, 19992a; Allen et al., 1991; Papahadjopoulos et al., 1991; Senior et al., 1991). Therefore, its effect on contents leakage was of interest. Due to its aqueous solubility (Lasic et al., 1991; Woodle et al., 1991) addition to pre-formed liposomes was carried out as for the block copolymers. The results demonstrate neither an increase in mean diameter nor leakage of the aqueous contents by addition of PEG-DSPE.

TABLE 2

Changes in mean diameter and % encapsulated ^{67}Ga-desferal from PC liposomes after addition of aqueous polymers

Added polymer		Mean diameter (nm)			% entrapped		
wt%	PEG-DSPE mol%	PEG-DSPE	Poly 908	F-127	PEG-DSPE	Poly 908	F-127
(9 · 1 mixture of liposome sample with aqueous polymer solution)							
0	0	88 5 ± 1 9	88 5 ± 1 9	88 5 ± 1 9	89 8	89.8	89.8
30	7.5	96.3 ± 0.6			89 4		
60	15	90.5 ± 1.0	90.2 ± 1.0	91.4 ± 1 2	89 3	83 8	25 1
100	20	91.5 ± 1 0	87.6 ± 1.5	92.1 ± 1 6	89 3	82.0	15 3

In order to investigate the influence of liposome charge and lipid composition on the adsorption and leakage, limited comparison studies with one polymer from each class, F-127, P-908, and PEG-DSPE, were performed with two neutral liposome preparations: one 'fluid' composed of PC and one 'rigid' composed of HSPC and cholesterol. The polymer F-127 was chosen for these studies since it appears from the results in Table 1 to exert the strongest action of the polymers in its class and thus is more likely to result in a more clearly measurable effect in analyses with different liposome compositions but should still be indicative of the other polymers in its class. The use of fluid liposomes composed of PC without charged lipids or cholesterol resulted in an increase in mean diameter with F-127 and PEG-DSPE of about 4 nm at a 100 wt% concentration (Table 2). Leakage of the aqueous contents, however, increased only with F-127. Similar results were obtained whether the liposomes were extruded or homogenized. An increase of about 6 nm in mean diameter was observed when F-127, and to a lesser extent PEG-DSPE, was added to liposomes composed of a high temperature phase transition lipid, fully hydrogenated soy PC (HSPC), and cholesterol resulting in more rigid bilayers (Table 3). In this case, however, leakage was reduced but not eliminated with F-127. Incorporation of PG into the HSPC:Chol liposomes eliminated the increase in mean diameter but had little effect on leakage of the aqueous contents (data not shown).

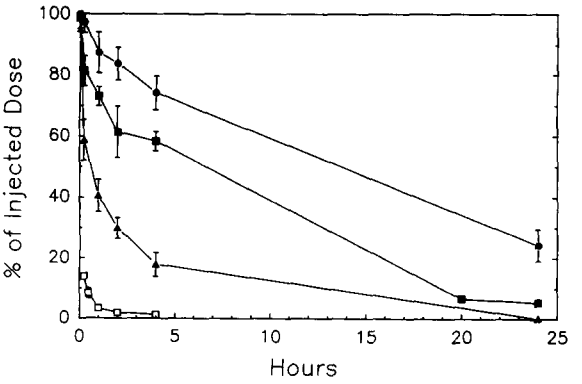

Fig. 2. Blood circulation in rats of ^{67}Ga-desferal labeled liposomes after i.v administration. (●) ^{1900}PEG-DSPE.PHEPC IV40:Chol at 0.15·1.85:1 mol ratio, (▲) EPG:PHEPC IV40:C at 0.15:1.85:1 mol ratio, (■) same EPG:PHEPC IV40:C as in the aforementioned treated with F-108, (□) aqueous ^{67}Ga-DF label. (mean ± S.D., $n = 3$)

In vivo blood circulation

The block copolymer F-108 adsorbed onto liposomes was examined for its effect on blood circulation in rats and compared with liposomes containing PEG-DSPE. The reasoning for the selection of F-108 for these studies was on the basis of, and for comparison with, a previous report indicating that this block copolymer should be preferred for liposomes since it showed reduced leakage when cholesterol was present in the liposomes (Jamshaid et al., 1988). The blood circulation kinetics in rats with F-108 adsorbed on liposomes are shown in Fig. 2 along with other results for comparison: free label, the PG:PC:

TABLE 3

Changes in mean diameter and % encapsulated ^{67}Ga-desferal from HSPC. Chol liposomes after addition of aqueous polymers

Added polymer		Mean diameter (nm)			% entrapped		
wt%	PEG-DSPE mol%	PEG-DSPE	Poly 908	F-127	PEG-DSPE	Poly 908	F-127
(9·1 mixture of liposome sample with aqueous polymer solution)							
0	0	99.7 ± 0.8	99.7 ± 0.8	99 7 ± 0.8	92.4	92.4	92.4
30	7.5	98.6 ± 1.4			94.3		
60	15	102.0 ± 1.0	98.5 ± 0.9	106.0 ± 1.0	92.8	93.5	65.1
100	20	103.0 ± 1.0	100.0 ± 0.9	106.0 ± 0.8	93 6	91.1	62 8

Chol liposomes before incubation with F-108, and PEG-DSPE-containing liposomes. These results demonstrate an increase in the blood circulation of these liposomes when F-108 is adsorbed. In comparison, in the case of the PEG-DSPE-containing liposomes further prolonged circulation was observed with its clearance following a single exponential process.

Discussion

These studies were performed in order to determine whether adsorption of block copolymers on liposomes provides a coating and the commensurate increase in blood circulation as found with latex and lipid emulsions. The results obtained were also compared with those observed with PEG-DSPE. The increase in mean diameter with three block copolymers was smaller than that reported in previous studies of up to 30 nm, representing a coating thickness up to 15 nm, with latex (Illum et al., 1986) and liposomes (Jamshaid et al., 1988; Moghimi et al., 1991).

The lack of an increase in mean diameter observed after addition of the block copolymers to the liposomes could be the result of little or no interaction with the liposomes. However, measures of entrapped label leakage provide strong evidence that the block copolymers interacted in a destructive manner with the liposomes as found previously (Jamshaid et al., 1988). All the block copolymers induced release of the label whereas the PEG-DSPE had no effect on any formulation. Alternatively, the coatings formed may have been of insufficient thickness to be readily measured by the method used, DLS. This is likely since the loss of the aqueous entrapped label observed differed according to the agent used, indicating that these materials interact with the liposomes to different extents. Perhaps, methods capable of measuring molecular interactions, such as through the use of radiolabels, influence of the surface coating on particle behavior, e.g., rotational motion or electrophoretic mobility (zeta-potential) due to hydrodynamic drag (Woodle et al., 1992b), or a procedure to separate the liposomes from polymer micelles, for example, by gel permeation chromatography or field-flow fractionation (Li and Caldwell, 1991) would permit a better measure of the interactions. Nevertheless, the question of whether or not the polymers interact, and if so how that interaction affects their properties, is the critical concern. The results obtained using DLS and measurement of the leakage of the aqueous contents clearly demonstrate that the polymers interact with the liposomes, albeit giving a surface coating with a thickness just at the limit of sensitivity of DLS. For these reasons, while further quantification of the adsorption of block copolymer onto the liposomes should contribute to a better understanding of the exact nature of the interactions, it should not substantially alter the conclusions drawn from these results. Nonetheless, investigations involving the use electrophoretic mobility and gel permeation are ongoing. In conclusion, strong evidence was obtained for interaction by the block copolymers with liposomes but forming a surface coating of only about 5 nm, roughly the limit of sensitivity of DLS.

The highest levels of the F-127 polymer resulted in a loss of 60% of the aqueous contents over 24 h with PG:PC:Chol liposomes. Previous observations of leakage were the motivation for the inclusion of cholesterol in the liposomes, since it is known to reduce both leakage in vivo (Kirby and Gregoriadias, 1981). It also has been suggested to reduce the interaction of block copolymers with liposomes (Jamshaid et al., 1988) and the results obtained here with liposomes lacking either cholesterol or PG demonstrated a slightly greater increase in both mean diameter and leakage on the addition of block copolymers. Surprisingly, a rigid liposome composed of a high temperature phase transition lipid in combination with cholesterol exhibited a similar increase in mean diameter but a reduction in leakage. Furthermore, addition of PG to these rigid liposomes eliminated the increase in mean diameter. These results suggest that the PG plays a greater role in reducing adsorption of block copolymers while the cholesterol affects leakage.

Despite the lack of an observed increase in mean diameter, adsorption of F-108 resulted in an increase in the blood circulation time com-

pared with the same liposomes without the block copolymer added (Fig. 1). However, this increase is substantially less than that observed when PEG-DSPE was incorporated: less than 1% to about 5% of the injected dose remaining in the blood after 24 h with F-108 compared to greater than 20% with PEG-DSPE. These results suggest that while some similarities may exist in the action of the block copolymers and PEG-PE on liposome blood circulation, quantitatively there is a substantial advantage of PEG-DSPE. Further investigation is needed in order to elucidate the details of the underlying mechanisms for these altered biological interactions and thereby identify methods capable of further advance in controlling liposome biodistribution.

References

Allen, T M. and Chonn, A., Large unilamellar liposomes with low uptake into the reticuloendothelial system *FEBS Lett*, 223 (1987) 42–46.

Allen, T M, Hansen, C., Martin, F, Redemann, C and Yau-Young, A Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half-lives in vivo *Biochim Biophys Acta*, 1066 (1991) 29–36

Bartlett, G.R, Phosphorus assay in column chromatography *J Biol Chem*, 234 (1959) 466–468.

Blume, G. and Cevc, G., Liposomes for the sustained drug release in vivo *Biochim Biophys Acta*, 1029 (1990) 91–97

Davis, S S and Hansrani, P, The influence of emulsifying agents on the phagocytosis of lipid emulsions by macrophages. *Int J Pharm*, 23 (1985) 69–77.

Fidler, I, Targeting of immunomodulators to mononuclear phagocytes for therapy of cancer. *Adv Drug Del Rev*, 2 (1988) 69–106.

Gabizon, A and Papahadjopoulos, D, Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors *Proc Natl Acad Sci USA*, 85 (1988) 6949–6953

Gabizon, A, Huberty, J., Straubinger, R.M., Price, D C and Papahadjopoulos, D, An improved method for in vVivo tracing and imaging of liposomes using a gallium 67-desferoxamine complex. *J Liposome Res.*, 1 (1988–89) 123–135.

Gregoriadis, G, *Liposomes as Drug Carriers· Recent Trends and Progress*, Wiley, New York, 1988

Illum, L and Davis, S.S., The organ uptake of intravenously administered colloidal particles can be altered using a non-ionic surfactant (poloxamer 338) *FEBS Lett.*, 167 (1984) 79–82.

Illum, L, Hunneyball, I.M. and Davis, S.S, The effect of hydrophilic coating on the uptake of colloidal particles by the liver and by peritoneal macrophages *Int J Pharm.*, 29, (1986) 53–65

Illum, L, Davis, S S, Muller, R H., Mak, E and West, P, The organ distribution and circulation time of intravenously injected colloidal carriers sterically stabilized with a block copolymer - poloxamine 908. *Life Sci.*, 40 (1987) 367–374

Illum, L, West, P., Washington, C and Davis, S.S. The effect of stabilising agents on the organ distribution of lipid emulsions. *Int. J. Pharm*, 54 (1989) 41–49

Jamshaid, M, Farr, S.J, Kearney, P. and Kellaway, I W, Polyoxamer sorption on liposomes comparison with polystyrene latex and influence on solute efflux. *Int J Pharm*, 48 (1988) 125–131.

Kirby, C. and Gregoriadias, G, Plasma-induced release of solutes from small unilamellar liposomes is associated with pore formation in the bilayers *Biochem J*, 199 (1981) 251–254.

Klibanov, A.L., Maruyama, K, Torchillin, V P and Huang, L., Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes *FEBS Lett*, 268 (1990) 235–237

Lang, J, Vigo-Pelfrey, C and Martin F J, Liposomes composed of partially hydrogenated egg phosphatidycholines fatty acid composition, thermal phase behavior and oxidative stability *Chem Phys Lipids*, 53 (1990) 91–101.

Lasic, D.D, Woodle, M.C, Martin, F J. and Valentincic, T, The structure of Stealth™ lipid-lecithin mixture *Period Biol*, 93 (1991) 287–290

Li, J T. and Caldwell, K.D, Sedimentation field-flow fractionation in the determination of surface concentration of adsorbed materials *Langmuir*, 7 (1991) 2034–2039

Moghimi, S M, Porter, C J.H, Illum, L. and Davis, S S., The effect of polyoxamer-407 on liposome stability and targeting to bone marrow comparison with polystyrene microspheres *Int J Pharm.*, 68 (1991) 121–126

Olson, F., Hunt, C A, Szoka, F C, Vail, W J. and Papahadjopoulos, D, Preparation of liposomes o defined size distribution by extrusion through polycarbonate membranes. *Biochim Biophys Acta*, 557 (1979) 9

Papahadjopoulos, D, Allen, T., Gabizon, A., Mayhew, E., Matthay, K, Huang, S K, Lee, K.-D, Woodle, M.C, Lasic, D.D, Redemann, C, and Martin, F J, Sterically stabilized liposomes pronounced improvements in blood clearance, tissue disposition, and therapeutic index of encapsulated drugs against implanted tumors *Proc Natl Acad Sci USA*, 88 (1991) 11460–11464

Riaz, M, Weiner, N and Martin, F, Liposomes. In Lieberman, H A, Rieger, M M. and Banker, G S. (Eds), *Pharmaceutical Dosage Forms. Disperse Systems*, Vol 2, Dekker, New York, 1988, pp.567–603

Senior, J H, Fate and behavior of liposomes in vivo a review of controlling factors *CRC Crit Rev Ther Drug Carrier Systems*, 3 (1987) 123–193

Senior, J, Delgado, C, Fisher, D, Tilcock, C and Gregori-

adis, G., Influence of surface hydrophilicity of liposomes on their interaction with plasma protein and clearance from the circulation: studies with poly(ethylene glycol)-coated vesicles. *Biochim. Biophys Acta,* 1062 (1991) 77–82

Sunamoto, J. and Iwamoto, K., Protein-coated and polysaccharide-coated liposomes as drug carriers. *CRC Crit Rev Ther Drug Carrier Systems,* 2 (1986) 117–136.

Torchilin, V.P, Berdichevsky, V.R., Barsukov, A.A. and Smirnov, V.N., Coating liposomes with protein decreases their capture by macrophages *FEBS Lett.*, 111 (1980) 184–188

Woodle, M.C. and Papahadjopoulos, D., Liposome preparation and size characterization *Methods Enzymol*, 171 (1988) 193–217

Woodle, M.C., Newman, M., Collins, L., Redemann, C. and Martin, F., Improved long circulating (Stealth®) liposomes using synthetic lipids. *Proc Int Symp Control Rel Bioact Mater*, 17 (1990) 77–78

Woodle, M.C., Lasic, D.D., Collins, L.R., Allen, T.M. and Martin, F.J., Phase diagram of PEG-DSPE (Stealth® lipid)-egg phosphatidylcholine (EPC) mixtures *Biophys J*, 59 (1991) 497a

Woodle, M.C., ^{67}Gallium-labeled liposomes with prolonged circulation· Preparation and potential as nuclear imaging agents. *Int J Nucl. Med. Biol*, (1993) in press

Woodle, M.C., Matthay, K.K., Newman, M.S., Hidayat, J., Collins, L.R., Redemann, C.R., Martin, F.J and Papahadjopoulos, D., Sterically stabilized liposomes versatility of lipid compositions with prolonged circulation *Biochim Biophys Acta,* 1105 (1992a) 193–200.

Woodle, M.C., Collins, L.R., Sponsler, E., Kossovsky, N., Papahadjopoulos, D and Martin, F.J., Sterically stabilized liposomes Reduction in electrophoretic mobility without change in electrostatic surface potential *Biophys J*, 61 (1992b) 902–910