Biochimica et Biophysica Acta, 1062 (1991) 142–148

ADONIS 000527369100108Z

BBAMEM 75131

Activity of amphipathic poly(ethylene glycol) 5000 to prolong the circulation time of liposomes depends on the liposome size and is unfavorable for immunoliposome binding to target

Aleksander L. Klibanov [1,2], Kazuo Maruyama [1], Anne Marie Beckerleg [1], Vladimir P. Torchilin [2] and Leaf Huang [1]

[1] *Department of Biochemistry, University of Tennessee, Knoxville, TN (U.S.A.) and* [2] *Department of Enzyme Engineering, Institute of Experimental Cardiology, Moscow (U.S.S.R.)*

(Received 16 August 1990)

Key words: Liposome; Circulation time; Poly(ethylene glycol); Targeted drug delivery; Drug targeting; Ganglioside

Dioleoyl-*N*-(monomethoxy polyethyleneglycol succinyl)-phosphatidylethanolamine (PEG-PE) (mol. wt. of PEG = 5000), an amphipathic polymer, can be incorporated into the liposome membrane and significantly prolong the blood circulation time of the liposome. As little as 3.7 mol% of PEG-PE in liposome resulted in maximal enhancement of liposome circulation time. However, this activity of PEG-PE was only seen with relatively small liposomes ($d \leq 200$ nm); larger liposomes containing PEG-PE showed an unusually high level (approx. 35% injected dose) of accumulation in the spleen. We have tested whether the small, PEG-PE containing liposomes are suitable for immuno targeting by incorporating a lung-specific monoclonal antibody on the liposome surface. While another amphiphile, ganglioside GM_1, which is well known for its activity to prolong the liposome circulation time, significantly enhanced the lung binding of the immunoliposomes, PEG-PE incorporation of immunoliposomes resulted in a low level of target binding. To test if the reduced target binding is due to a steric barrier effect of the surface PEG polymer, we have incorporated a small amount of *N*-biotinaminocaproylphosphatidylethanolamine into the PEG-PE containing liposomes and examined the liposome agglutination induced by the addition of streptavidin. As little as 0.72 mol% PEG-PE in these liposomes completely abolished agglutination. In contrast, incorporation of GM_1 in liposomes only reduced the rate, but not the extent, of liposome agglutination. These results strongly support the hypothesis that PEG-PE prolongs liposome circulation time by providing a strong steric barrier which prevents close contact with another liposome or cell. Since GM_1 provides only a weak steric barrier effect, its activity to prolong the liposome circulation time must involve another yet unknown mechanism.

Introduction

Some of the most significant progress in the study of liposomal drug delivery is in the recent development of liposomes with reduced affinity to the reticuloendothelial system. These vesicles exhibit a prolonged circulation time in the blood [1]. Gabizon and Papahadjopoulos [2] have demonstrated that these new liposomes accumulate to significantly higher levels in solid tumors than do conventional liposomes. We have shown that a prolonged circulation time of liposomes also enhances the target binding and retention of the liposomes bearing target-specific monoclonal antibodies [3]. Most of these experiments were done with the ganglioside GM_1 as the necessary lipid component which endows the liposomes with the activity of prolonged circulation. Recently, we [4] and others [5] have shown that amphipathic PEG such as PEG conjugated to PE can also significantly prolong the circulation time of liposomes if the conjugate is incorporated into the liposome membrane. The

Abbreviations: biotin-cap-PE, biotinamidocaproyl-PE; chol, cholesterol; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylethanolamine; DTPA-SA, distearylamide of diethylenetriaminepentaacetic acid; NGPE, *N*-glutaryl-PE; PC, egg yolk phosphatidylcholine; PE, dioleoylphosphatidylethanolamine; PEG, poly(ethylene glycol); PEG-PE, dioleoyl-*N*-(monomethoxypolyethyleneglycol-succinyl)-PE; PG, egg phosphatidyl-DL-glycerol; PS, bovine brain phosphatidylserine; SM, sphingomyelin.

Correspondence: L. Huang, Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840, U.S.A.

activity of PEG-PE to prolong the liposome circulation time is greater than that of GM_1 on a molar basis [4].

In this report we describe the characterization of PEG-PE and further investigate the biodistribution of these PEG-PE containing liposomes. We have specifically investigated whether the immunoliposomes containing PEG-PE bind efficiently to their target. The steric barrier mechanism of PEG on the liposome surface is also demonstrated using a biotin-avidin mediated liposome agglutination assay. These studies have shed some light on the suitability of the PEG-PE containing liposomes for the various applications of drug delivery.

Experimental procedures

Materials

Cholesterol, streptavidin, and sulforhodamine B were purchased from Sigma. Ganglioside GM_1 was purchased from Calbiochem. DOPE, NGPE, DPPC, DSPC, SM, PS, PG and PC from Avanti Polar Lipids were used. $^{111}InCl_3$ (carrier-free) was from New England Nuclear. Synthesis of DTPA-SA has been described [6] and so has the synthesis of biotin-cap-PE [7]. PEG-PE (mol. wt. of PEG = 5000) was synthesized as described [4] except that the PEG-PE was purified by prolonged dialysis of an aqueous suspension of the reaction mixture using a dialysis bag with large pores (Spectra-Por CE, 300000 MWCO, Spectrum Medical Industries). [^{14}C]PEG-PE was synthesized by using [^{14}C]DOPE which was obtained from Amersham International. Monoclonal antibody 34A directed against mouse lung endothelial cells was isolated essentially as described earlier [8].

Labeling of DTPA-SA with ^{111}In

Radiolabeling of DTPA-SA with ^{111}In was performed in ethanol. 50 μl of 0.1 mg/ml solution of DTPA-SA in warm ethanol was mixed with 3 μl of the $^{111}InCl_3$ solution in 0.01 M HCl (5–50 μCi). 50 min later, 0.5 μl triethylamine was added and the mixture was incubated at room temperature for 1 h before it was used for labeling the liposomes.

Liposome preparation

Unless otherwise stated, liposomes were prepared from PC and cholesterol (1 : 1, molar ratio) with a tracer marker ^{111}In-DTPA-SA. In some cases, 7.4 mol% of ganglioside GM_1 or PEG-PE was also added to the lipid mixture. Each mixture of lipids in $CHCl_3$/EtOH was placed in a round-bottom glass tube and the organic solvent was evaporated with a stream of nitrogen gas. The lipid film was additionally vacuum desiccated for 2 h. Normal saline was added and the lipid was allowed to hydrate for 1 h at room temperature. The lipid suspension was vortexed for 2 min and extruded ten times through two stacked Nuclepore polycarbonate filters (in most cases, 0.2 μm pores; for the size dependence experiment stacked 0.1 μm, 0.2 μm, 0.4 μm, 0.8 μm filters were used). Liposome size was estimated with a Coulter N4SD sub-micron particle analyzer (Hialeah, FL). In case of SM, DPPC, DSPC-containing liposomes, extrusion was performed at 60°C in a thermostat-heated, high-pressure extrusion devise (Lipex, Vancouver, Canada). All lipid compositions are given as molar ratios unless otherwise indicated.

Radiolabeling of proteins

Radiolabeling of proteins with ^{125}I was performed via the Iodo-Gen procedure [9].

Immunoliposome preparation

Immunoliposomes bearing 34A monoclonal antibody were prepared essentially as described previously [10]. 34A with a trace amount of ^{125}I-labeled 34A was first conjugated to phospholipid using the NGPE reagent [10]. The antibody-NGPE conjugate was then added to a mixture of lipids (trace labeled with ^{111}In-DTPA-SA) which had been solubilized with the detergent octyl glucoside. The input weight ratio 34A-to-lipid was 1 : 2 for the PEG-PE containing immunoliposomes, and 1 : 4 for the other immunoliposome preparations. After a dialysis procedure to remove the detergent, immunoliposomes were extruded through a stack of 2 Nuclepore filters (0.2 μm) and the unbound antibody removed by gel filtration on a Bio-Gel A15M column. The protein-to-lipid ratio of the resulting immunoliposomes was calculated from the ^{125}I-cpm/^{111}In-cpm ratio, corrected according to the specific radioactivity of each labeled compound.

Biodistribution studies

Biodistribution studies were performed on male Balb/c mice weighing 18–23 g. Liposomes labeled with ^{111}In were injected in the tail vein. After a specified period of time, animals were anesthetized with Metofane (Pitman-Moore, NJ) and killed by cervical dislocation. Samples of blood and internal organs were collected, weighed and ^{111}In radioactivity was determined in a Beckman gamma counter. Weight of mouse blood was assumed to be 7.3% of the body weight [11]. Data were expressed as percent of the injected dose accumulated per organ.

Liposome agglutination by streptavidin

Liposomes used in this experiment were prepared from PC/chol (1 : 1) and contained additionally 2.5% biotin-cap-PE and varying amount of GM_1 or PEG-PE. Fifty μl liposomes (50 μg phospholipid) were mixed with 0.5 ml PBS in a microcuvette, and 5–10 μg streptavidin was added. Increase in turbidity was monitored as the optical density at 440 nm.

Binding of ^{125}I-streptavidin to biotinylated liposomes

Liposomes used in this experiment were prepared from PC/chol (1 : 1) and contained additional 2.5% biotin-cap-PE and varying amounts of GM_1 or PEG-PE. 100 μl liposomes (10 μg phospholipid in PBS) were mixed with 0.2 μg ^{125}I-streptavidin and incubated at room temperature for 10 min. Unbound streptavidin was separated from the liposomes by gel filtration on Bio-Gel A1.5M. ^{125}I cpm in the liposome fractions was taken as the amount of ^{125}I-streptavidin bound to liposomes.

Results

Incorporation of PEG-PE into the liposome membrane

The PEG-PE conjugate was amphipathic as it readily formed micellar aggregates in aqueous solution. [^{14}C]PEG-PE was mixed with a water-soluble dye, sulforhodamine B, and the mixture was chromatographed on a Bio-Gel A50M column. Both molecules were eluted in the included volume fractions but were well separated from each other (Fig. 1B). In a parallel experiment, [^{14}C]PEG-PE was incorporated into liposomes. The [^{14}C]PEG-PE was eluted in the void volume fractions together with liposomes (Fig. 1A). Furthermore, a portion of sulforhodamine B was co-eluted in the liposome fractions as the entrapped solute. These results indicate the amphipathic nature of PEG-PE and that its incorporation into the liposome membrane does not interfere with entrapment of an aqueous marker.

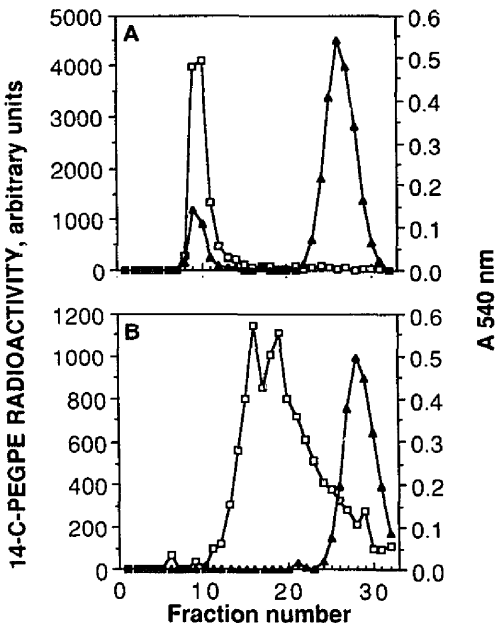

Fig. 1. Gel-filtration of PEG-PE-containing liposomes or PEG-PE micelles. Trace [^{14}C]PEG-PE was added to the unlabeled PEG-PE which was incorporated into liposomes (A), or dispersed in the normal saline by itself (B), in the presence of sulforhodamine B as an aqueous marker. The mixtures were gel-filtered on a Bio-Gel A50M column. ^{14}C radioactivity (□) and the optical density (A_{540} nm) of sulforhodamine B (▲) of each fraction are plotted.

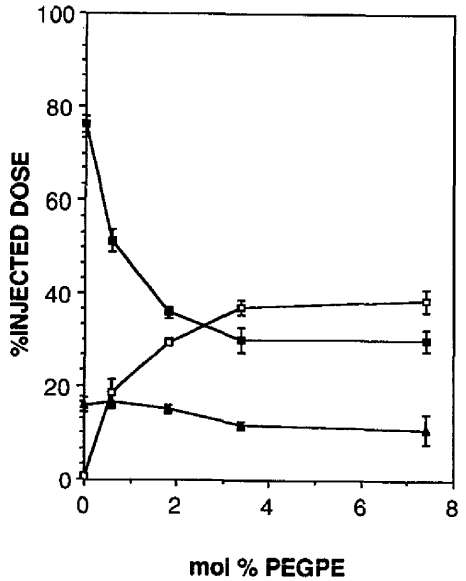

Fig. 2. The effect of PEG-PE on the biodistribution of liposomes (PC/chol = 1 : 1) in mouse. ^{111}In-labeled liposomes ($d = 208 \pm 40$ nm) containing various amounts of PEG-PE were i.v. injected into mice and the % injected dose was measured in blood (□), liver (■) and spleen (▲) 5 h after injection. Data were obtained from 3 mice/group and shown as mean ± S.D.

Effect of PEG-PE on the liposome biodistribution

Homogeneous, ^{111}In-labeled liposomes with an average diameter of 192 ± 6 nm were prepared by the extrusion method. These liposomes, containing various amounts of PEG-PE, were injected i.v. into mice via the tail vein and the biodistribution was measured 5 h later. Fig. 2 shows the % of the injected dose in blood, liver and spleen as a function of the PEG-PE content of the liposomes. It is clear that an increase in the PEG-PE concentration in liposomes resulted in an increase of liposomes in the blood compartment, and a concomitant decrease of the liposomes in the liver and spleen. The effect of PEG-PE showed a plateau at a PEG-PE concentration of 3.7%. These data clearly indicate the activity of PEG-PE to prolong the circulation time of these liposomes and to decrease the uptake of liposomes by the reticuloendothelial system.

Dependence of liposome biodistribution on the liposome size

The above experiment was done with liposomes of a relatively small diameter ($d \approx 200$ nm). The effect of liposome size on the biodistribution of liposomes containing PEG-PE was studied (Fig. 3). Surprisingly, the high level of liposomes in the blood was only observed for the small liposomes ($d \lesssim 200$ nm); larger liposomes ($d > 200$ nm) showed a high level of accumulation in the spleen and a low concentration in blood. The liver uptake of the PEG liposomes was not dependent on the liposome size. The accumulation of such an unusually high level (approx. 35% injected dose) of the large liposomes in the spleen was unique to this kind of liposome; large liposomes containing no PEG-PE did not accumulate significantly in the spleen (data not shown). Thus, the effect of PEG-PE to prolong liposome circulation time is limited to smaller liposomes. Larger liposomes containing PEG-PE were still cleared from the circulation, but accumulated in the spleen in addition to the liver.

Effect of lipid composition on the blood clearance of liposomes containing PEG-PE

The activity of prolonging blood circulation time of liposomes depends on the lipid composition [2,12]. We have varied the lipid composition of liposomes of a constant diameter ($d \approx 200$ nm) containing a constant amount (7.4%) of PEG-PE. Table I shows the percent of the injected liposomes in the blood 5 h after i.v. injection. Compared to the basic composition of PC/chol (1 : 1), other lipid compositions tested gave very similar results for the amount of liposomes in the blood, except the liposomes containing PC/chol/PS. Thus, PEG-PE's activity to prolong liposome circulation time is compatible with both fluid (PC/chol, PC/chol/GM_1, PC/chol/PG) and solid (SM/PC/chol, DPPC/chol, DSPC/chol) lipid compositions. There is no synergistic effect of PEG-PE and GM_1, since PC/chol liposomes with or without GM_1 showed similarly high blood concentrations. The low level of liposomes composed of PC/chol/PS in the blood indicated that PEG-PE's activity to prolong circulation time was completely inhibited by the presence of PS in liposomes. This inhibitory effect of PS was not due to its negative charge content, since the liposomes containing the negatively charged lipids, PG and GM_1, showed high blood concentrations (Table I).

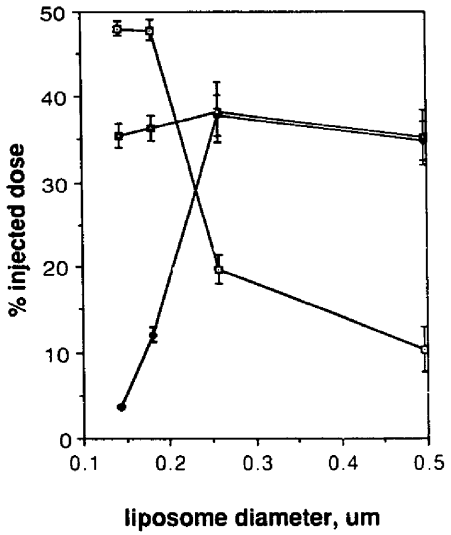

Fig. 3. Dependence of biodistribution of PEG-PE containing liposomes on the liposome size. ^{111}In-labeled liposomes (containing 7.4% PEG-PE) of various diameters were i.v. injected into mice. The % injected dose was measured in blood (□), liver (■) and spleen (▲) 5 h after injection. Data were obtained from 3 mice/group and shown as mean ± S.D.

TABLE I

Effect of lipid composition on the blood concentration of PEG-PE containing liposomes

Lipid composition	Blood concentration (% injected dose)
PC/chol/PEG-PE (1 : 1 : 0.15)	47.8 ± 1.2 [b]
SM/PC/chol/PEG-PE (1 : 1 : 1 : 0.15)	43.9 ± 4.4
DPPC/chol/PEG-PE (1 : 1 : 0.15)	46.7 ± 1.7
DSPC/chol/PEG-PE (1 : 1 : 0.15)	39.0 ± 2.1
PC/chol/GM_1/PEG-PE (1 : 1 : 0.15 : 0.15)	52.1 ± 0.9
PC/chol/PG/PEG-PE (1 : 1 : 0.15 : 0.15)	40.8 ± 2.7
PC/chol/PS/PEG-PE (1 : 1 : 0.15 : 0.15)	0.6 ± 0.1

^{111}In-labeled liposomes (approx. 200 nm in diameter) of the indicated lipid composition were i.v. injected into mice. The % of injected dose remaining in the blood was measured 5 h after injection.

[b] Mean ± S.D. ($n = 3$).

Effect of PEG-PE and GM_1 on target binding of immunoliposomes

To test whether the enhanced circulation time of liposomes will facilitate the binding of immunoliposomes to a target, we have incorporated a monoclonal antibody-phospholipid conjugate into the liposome membrane. Monoclonal antibody 34A is a lung-specific antibody which binds with a glycoprotein antigen, gp112, on the lumenal surface of the capillary endothelial cells of the mouse lung [8]. Immunoliposomes containing 34A rapidly localize in the lung after i.v. injection [3,10]. We have prepared an antibody-NGPE conjugate and incorporated it into small liposomes ($d \approx$ 200 nm) composed of PC/chol (1 : 1) or PC/chol (1 : 1) additionally containing 4 mol% of either GM_1 or PEG-PE. These immunoliposomes contained similar amounts of the antibody-NGPE conjugate. The weight ratio of

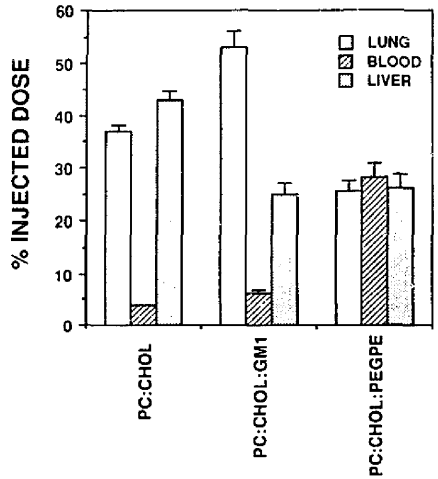

Fig. 4. Target binding of immunoliposomes containing PEG-PE or GM_1. ^{111}In-labeled immunoliposomes ($d \approx 200$ nm) containing the lung-specific monoclonal antibody 34A were injected i.v. and the % injected dose was measured in lung (open bars), blood (hatched bars) and liver (shaded bars) 1 h after injection. Lipid composition and the protein-to-lipid ratio (by weight) of immunoliposomes were: (1) PC/chol (1 : 1), 1 : 36; (2) PC/chol/GM_1 (1 : 1 : 0.15), 1 : 37, and (3) PC/chol/PEG-PE (1 : 1 : 0.085), 1 : 30.

protein-to-lipid of the three liposome preparations ranged from 1 : 30 to 1 : 37. The biodistribution of these immunoliposomes in the mouse were measured 1 h after i.v. injection. Data shown in Fig. 4 show that the presence of GM_1, which greatly prolongs the liposome circulation time [1,2,12,13], significantly enhanced lung binding (from 37% to 52%) and decreased the liver uptake (from 42% to 25%) of the immunoliposomes. However, the presence of PEG-PE decreased both lung binding (from 37% to 26%) and liver uptake (from 42% to 25%) of immunoliposomes. The blood concentration of immunoliposomes was the highest for liposomes containing PEG-PE (approx. 27%). Low levels of the injected dose were found in the blood for liposomes containing PC/chol and PC/chol/GM_1. These results clearly indicate the opposite effects of GM_1 and PEG-PE in terms of the target binding of immunoliposomes. GM_1 promoted target binding and decreased liver uptake of immunoliposomes; however, PEG-PE reduced both target binding and liver uptake, resulting in a high level of the injected dose remaining in the blood.

Inhibition of liposome agglutination by PEG-PE and GM_1

One of the possibilities that PEG-PE inhibits both the reticuloendothelial system uptake and the target binding of the immunoliposomes is due to a strong steric barrier effect provided by the relatively bulky PEG polymers on the liposome surface. To test this hypothesis in a defined model system, we have studied the agglutination induced by streptavidin of liposomes containing biotin-cap-PE. Streptavidin is a tetrameric protein which binds with the liposome surface biotin with a high affinity [14] and promotes a rapid agglutination of liposomes as measured by turbidity increase of the liposome suspension (Fig. 5). The presence of PEG-PE in liposomes, as little as 0. 72% of the total lipids, completely prevented liposome agglutination induced by streptavidin. These results indicate that even a small amount of the PEG polymer in liposomes can inhibit the close contact among the liposomes which is required for liposome agglutination. In contrast to these results, a parallel liposome agglutination experiment was done with the liposomes containing GM_1 (Fig. 6). There was a GM_1 concentration-dependent inhibition of the rate, but not the extent, of liposome agglutination. Furthermore, the inhibition of the liposome agglutination rate was not pronounced until the GM_1 concentration was more than 6%. It is clear from these data that GM_1, unlike PEG-PE, presents only a weak steric barrier for liposome agglutination.

Binding of streptavidin to liposomes containing biotin-cap-PE

To see if the absence of the liposome agglutination for the liposomes containing PEG-PE was due to the

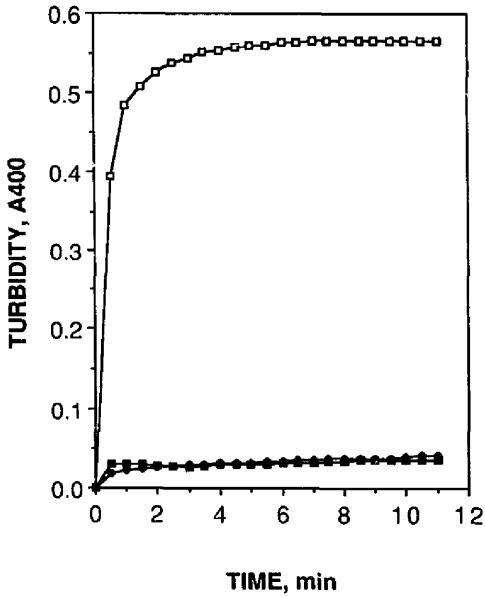

Fig. 5. Effect of PEG-PE on the streptavidin-induced agglutination of liposomes containing biotin-cap-PE. Increase of the turbidity ($A_{440\,nm}$) was measured with time for liposomes containing 0% (□), 0.72% (■) and 7.4% (◆) PEG-PE.

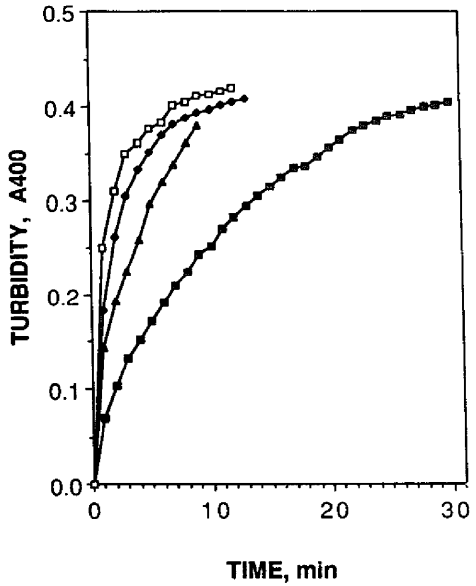

Fig. 6. Effect of GM_1 on the streptavidin-induced agglutination of liposomes containing biotin-cap-PE. Increase of turbidity (A_{440nm}) was measured with time for liposomes containing 0% (□), 2% (◆), 6% (▲) and 10% (■) GM_1.

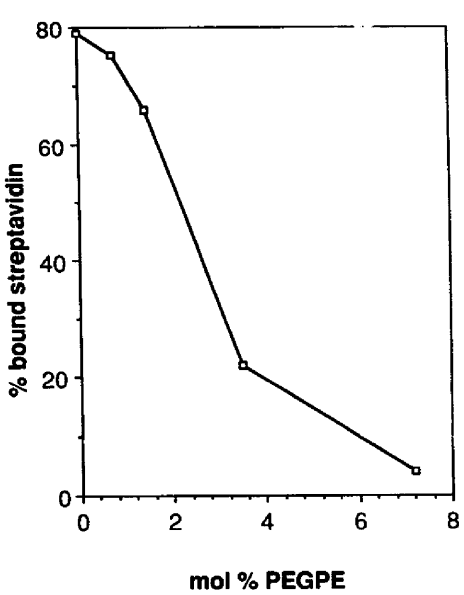

Fig. 7. Effect of PEG-PE on the binding of streptavidin with liposomes containing biotin-cap-PE. ^{125}I-streptavidin (2 μg/ml) was incubated with liposomes containing various amounts of PEG-PE for 10 min at room temperature. The mixtures were gel-filtered on a Bio-Gel A1.5M column to separate liposomes from the unbound streptavidin. The % bound streptavidin was plotted against the amount of PEG-PE in liposomes.

lack of streptavidin binding to liposomes, we have used ^{125}I-streptavidin and directly measured its binding to liposomes containing various amounts of PEG-PE (Fig. 7). Streptavidin binding to liposomes containing biotin-cap-PE was inhibited by PEG-PE in a concentration-dependent manner. However, no significant inhibition occurred at PEG-PE concentrations less than 2%. These data indicate that while at low PEG-PE concentrations the binding of a protein molecule to the liposome surface is not significantly inhibited, close apposition of liposomes among themselves is severely blocked at these low concentrations. In other words, low levels of PEG polymer on the liposome surface do not prevent the approach of a protein molecule, but hinder the approach of a larger surface such as another liposome. At higher concentrations of PEG-PE, both events are blocked.

Discussion

We have shown previously that incorporation of PEG-PE conjugates can effectively prolong the circulation time of liposomes [4]. The present study shows that PEG-PE only prolongs the circulation time of the relatively small liposomes ($d \leq 200$ nm). Larger liposomes were still cleared from the blood and accumulated in the spleen of the animal. It is important to note that the liver uptake of the PEG-PE containing liposomes was not size-dependent; it remained at about 35–40% of injected dose for liposomes with diameters ranging from 0.1 to 0.5 μm. This result shows that the presence of the PEG polymer on the liposome surface effectively decreases the uptake of liposomes by the Kupffer cells in the liver, but it has increased the chance of the liposomes encountering the spleen. It is known that only a small fraction (less than 7%) of the total blood flow circulates through the spleen [15]. The major function of this organ is to filter the aged or damaged red blood cells. By decreasing the liver uptake of liposomes, which appears to be the primary function of the surface PEG, liposomes enter the spleen more frequently and are more susceptible to the filter mechanism of the organ. Whether the large liposomes retained in the spleen are phagocytosed by the splenic macrophages like aged and damaged red blood cells is not clear at present. The steric barrier presented by the PEG polymer may also prevent the uptake of liposomes by splenic macrophages. Preliminary data from this laboratory indicate that large liposomes containing GM_1 also accumulate in the spleen (Liu, Mori and Huang, unpublished observation).

It is not surprising to see that the activity of PEG-PE in prolonging the blood circulation time of liposomes works on liposomes of diverse lipid compositions. Liposomes composed of lipids with high and low chain melting temperatures, or with neutral or negative charges

show the same high levels of blood concentrations (Table I). However, the presence of PS strongly antagonized the effect of PEG-PE. PS is well known for its activity in promoting liposome uptake by the reticuloendothelial system [16]. It is puzzling to see that PS can somehow obliterate the strong steric barrier provided by PEG. One possibility is that liposomes undergo a phase separation in the presence of the divalent cations such as calcium in the blood [18,19] and the domains enriched with PS-divalent cation complexes (but excluding PEG-PE) readily attract the alleged opsonin molecules from the serum [17], resulting in a rapid uptake of liposomes by the reticuloendothelial system. Further experimentation is necessary to test this hypothesis.

Data in Fig. 4 and our previous results [3] clearly show that GM_1 can significantly enhance the target binding of immunoliposomes, however, PEG-PE actually inhibits it. Both GM_1 and PEG-PE can significantly prolong the blood circulation time of liposomes [4,5]. These results can be explained in view of the strong steric barrier activity of the PEG as shown by the model studies of liposome agglutination (Fig. 5). Surface PEG polymers may present a steric hindrance for binding of liposome bound antibodies with target antigens on endothelial cell surfaces. GM_1 is a much weaker steric barrier as shown by the data in Fig. 6. The presence of GM_1 on liposome surfaces is apparently sufficient to prevent a rapid uptake by macrophages, but not to provide a hindrance for antibody-antigen binding. In fact, the elevated blood concentration of liposomes containing GM_1 has kinetically enhanced the target binding of immunoliposomes [3], but the same effect provided by PEG-PE does not lead to an enhanced target binding. These results clearly indicate that the amphipathic PEG molecules described here are not suitable for the targeted drug delivery by liposomes. However, it is conceivable that PEG molecules of shorter chain length (the one used in the present study has a mol. wt. of 5000) may present a weaker steric barrier for the target binding of immunoliposomes. Alternatively, longer spacer arm could be used to link the antibody molecules to the liposome surface to overcome the steric barrier of PEG. These possibilities will be tested in future experiments. Although PEG-PE may not be useful in immunoliposome targeting, its use in prolonging the circulation time of liposomes should not be overlooked. Water-soluble drugs entrapped in these liposomes may be released over a prolonged period of time to achieve a sustained high concentration of the drug in the blood. Such improved pharmacokinetics is likely to enhance the therapeutic efficacy of the drug.

In summary, we have described some detailed biodistribution experiments which have characterized the activity of PEG-PE incorporated into the liposome and immunoliposome membranes. The activity in prolonging the circulation time of liposomes is limited to the relatively small liposomes ($d \leq 200$ nm), since larger liposomes are taken up by the spleen probably with a filter mechanism. The strong steric barrier activity of PEG-PE on the liposome surface, documented by a model study of liposome agglutination, is likely to give some limit to the use of the PEG-PE containing liposomes in drug delivery.

Acknowledgment

This work was supported by NIH grants CA24553 and AI25834, and is benefitted by the US-USSR Medical Exchange Program in the Cardiovascular Area. We thank Stephen Kennel for providing the 34A antibody and a critical review of the manuscript, Carolyn Drake for help in word processing, and Dexi Liu and Atsu Mori for sharing their unpublished data. Liu and Mori were the first to observe the spleen accumulation of the long-circulating liposomes.

References

1 Allen, T. M. and Chonn, A. (1987) FEBS Lett. 223, 42–46.
2 Gabizon, A. and Papahadjopoulos, D. (1988) Proc. Natl. Acad. Sci. USA 85, 6949–6953.
3 Maruyama, K., Kennel, S. and Huang, L. (1990) Proc. Natl. Acad. Sci. USA, 87, 5744–5748.
4 Klibanov, A.L., Maruyama, K., Torchilin, V.P. and Huang, L. (1990) FEBS Lett., 268, 235–237.
5 Senior, J., Delgado, C., Fisher, D. and Gregoriadis, G. (1990) Abstracts of Conference "Liposome Research Days", University of Florida, Gainesville, FL, Feb. 28–March 2, 1990.
6 Kabalka, G.W., Buonocore, E., Hubner, K., Moss, T., Norley, N. and Huang, L. (1987) Radiology 163, 255–258.
7 Bayer, E.A., Rivnay, B. and Skutelsky, E. (1979) Biochim. Biophys. Acta 550, 464–473.
8 Kennel, S. J., Lankford, T., Hughes, B. and Hotchkiss, J. (1988) Lab. Invest. 59, 692–701.
9 Catalog of Pierce Chemical Co. (1989) p. 258.
10 Holmberg, E., Maruyama, K., Litzinger, D., Wright, S., Davis, M., Kabalka, G.W., Kennel, S.J. and Huang, L. (1989) Biochem. Biophys. Res. Commun. 165, 1272–1278.
11 Wu, M.S., Robbins, J.C., Bugianesi, R.L., Ponpipom, M.M. and Shen, T.Y. (1981) Biochim. Biophys. Acta 674, 19–26.
12 Allen, T.M., Hansen, C. and Rutledge, J. (1989) Biochim. Biophys. Acta 981, 27–35.
13 Liu, D.X. and Huang, L. (1990) Biochim. Biophys. Acta 1022, 348–354.
14 Hashimoto, K., Loader, J.E. and Kinsky, S. (1986) Biochim. Biophys. Acta 856, 556.
15 Vick, R.L. (1984) Contemporary Medical Physiology, Chap. 72, p. 521, Addison-Wiley, Menlo Park, CA.
16 Allen, T. M., Williamson, P. and Schlegel, R.A. (1988) Proc. Natl. Acad. Sci. USA 85, 8067–8071.
17 Moghimi, S.M. and Patel, H.M. (1989) Biochim. Biophys. Acta 984, 379–383.
18 Ohnishi, S. and Ito, T. (1973) Biochem. Biophys. Res. Commun. 51, 132–138.
19 Hui, S.W., Boni, L.T., Stewart, T.P. and Isac, T. (1983) Biochemistry 22, 3511–3516.