ELSEVIER

International Journal of Pharmaceutics 115 (1995) 183–191

international journal of pharmaceutics

Disposition kinetics of liposomes modified with synthetic aminoglycolipids in rats

Hiromitsu Aoki [a,*], Changqi Sun [a], Kaoru Fuji [b], Koichiro Miyajima [a]

[a] *Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan*
[b] *Institute for Chemical Research, Kyoto University, Uji, Kyoto, Japan*

Received 21 April 1994; modified version received 16 July 1994; accepted 28 July 1994

Abstract

Positively charged methyl-2-amino-6-palmitoyl-D-glycosides (PGLNs), i.e., derivatives of glucose (PGlcN), galactose (PGalN) and mannose (PManN), were synthesized to replace toxic stearylamine (SA) and were incorporated into liposomes. Methyl-6-palmitoyl glycosides (PGLs), namely, derivatives of glucose (PGlc), galactose (PGal) and mannose (PMan), were also synthesized for comparison with PGLN liposomes. PGLN and PGL liposomes newly prepared by extrusion were used to investigate their disposition kinetics in rats. The zeta potentials of liposomes, all of which contain 20 mol% of PGLN and have a diameter of about 200 nm, were about 10 mV in PBS. Those of egg PC/cholesterol and PGLN liposomes had small negative zeta potentials. These PGLN liposomes showed no hemolytic activity and no toxicity in the experimental concentration range. PGLN and PGL liposomes have the same stability as egg PC/cholesterol liposomes in rat serum. PGLNs prolonged the elimination of liposomes from the blood circulation and decreased the hepatic uptake of liposomes, while PGLs showed no effects. It was suggested that the positive charge on PGLNs is one important factor for the prolongation of the residency of liposomes in the blood circulation.

Keywords: Liposome; Aminoglycolipid; Positive charge; Reticuloendothelial system; Blood cell

1. Introduction

Liposomes have so far been studied as both biomembrane models and as carrier systems for drug delivery. Liposomes have various advantages as drug carriers, being biodegradable, having low toxicity and being able to encapsulate hydrophilic, lipophilic and amphipathic drugs. However, there are inevitable drawbacks to their use in vivo. Liposomes are recognized as foreign substances and phagocytosed by cells of the reticu-

Abbreviations: EPC, egg yolk phosphatidylcholine; Chol, cholesterol; SA, stearylamine; PGLN, 2-amino-6-palmitoylglycoside; PGlcN, methyl-2-amino-2-deoxy-6-*O*-palmitoyl-D-glucoside; PGalN, methyl-2-amino-2-deoxy-6-*O*-palmitoyl-D-galactoside; PManN, methyl-2-amino-2-deoxy-6-*O*-palmitoyl-D-mannoside; PGL, 6-palmitoylglycoside; PGlc, methyl-6-*O*-palmitoyl-D-glucopyranoside; PGal, methyl-6-*O*-palmitoyl-D-galactopyranoside; PMan, methyl-6-*O*-palmitoyl-D-mannopyranoside; LacCer, lactosylceramide; TMS, tetramethylsilane.

* Corresponding author. Tel. (075) 753-4574; Fax (075) 761-2698.

SSDI 0378-5173(94)00252-5

loendothelial system (RES). The rapid removal from the circulation following intravenous administration is primarily due to phagocytosis by the Kupffer cells and macrophages of the spleen (Gregoriadis and Allison, 1980; Gregoriadis, 1988). Native liposomes are especially suitable for targeting diseases of the RES such as leishmaniasis and fungal infections (Alving et al., 1978; Chance et al., 1978) but are undesirable for delivering drugs to other organs.

There are many factors affecting the fate of liposomes in vivo, including lipid composition, liposome size, surface charge, lipid fluidity, etc. For example, small unilamellar vesicles (SUVs) are taken up less rapidly than large ones (Senior et al., 1985). Incorporation of cholesterol (Chol) into liposomes increases the stability of liposomes in the serum and reduces the clearance rate from the blood circulation (Senior and Gregoriadis, 1982). Coating liposomes with polysaccharides, particularly ganglioside G_{M1}, can lead to a prolonged lifetime in the circulation due to low uptake by the RES. Sialic acid plays an important role in the process of uptake of G_{M1} liposomes by the RES. However, G_{M2} and G_{M3}, which also have a sialic group, have no such RES avoiding nature (Allen and Chonn, 1987). Positively charged SA liposomes also remain in the blood longer than neutral or acidic liposomes (containing phosphatidylserine or phosphatidic acid) (Gregoriadis and Neerunjun, 1974), but SA is difficult to apply therapeutically because of its toxicity (Adams et al., 1977; Mayhew et al., 1987). In another approach to avoid the phagocytosis of macrophages, coating with chains of polyethylene glycol (PEG) can lead to a prolonged circulation time of liposomes due to steric hindrance or increase in liposomal surface hydrophilicity (Illum and Davis, 1984; Allen et al., 1991; Woodle et al., 1992; Blume and Cevc, 1993).

In this study, we synthesized several biodegradable monoesters of fatty acid with amino sugars and neutral sugars. We prepared liposomes coated with amino and neutral sugars, then investigated the effects of the positive charge and the sugar structure on the disposition property of liposomes in rat.

2. Materials and methods

2.1. Material

Egg yolk L-α-phosphatidylcholine (EPC) was obtained from Asahi Kasei Co. Ltd (iodine value 65, Japan). Stearylamine (SA), lactosylceramide (LacCer) and 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) were purchased from Sigma Chemical Co. (U.S.A.). Calcein (3,3′-bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein) was supplied by Dojin (Japan). [1,2(n)-^{3}H]Cholesteryl hexadecyl ether, [^{3}H]CHE was purchased from Daiichi Pure Chemical Co. Ltd. (Japan). Soluene-350® was purchased from Packard Instrument Co. Inc. (U.S.A.). Clear-sol I was obtained from Nacalai Tesque Inc. (Japan). All other chemicals from Wako Pure Chemical Ind. Ltd (Japan) were of special grade and cholesterol (Chol) was used after recrystallization from ethanol. Water was glass distilled twice.

2.2. Synthesis of methyl-2-amino 6-palmitoylglucoside

Methyl-2-amino-6-palmitoyl-D-glucoside (PGlcN, Fig. 1) was synthesized in a similar manner to that described by Miyajima et al. (1993). The synthesized compound was characterized by measurement of its ^{1}H-NMR spectrum in DMSO-d_6 using TMS as a reference on a spectrometer (JMN-GX400, JEOL), by elemental analysis, infrared and mass spectra. The positions of the esters were determined by 2D H-H COSY. ^{1}H-NMR 0.86 (t, $J = 6.0$ Hz, 3H), 1.24 (b, 24H), 1.51 (m, 2H), 2.29 (t, $J = 7.2$ Hz, 2H), 2.40 (m, 1H), 3.10 (m, 2H), 3.26 (s, 3H), 3.53 (m, 1H), 4.04

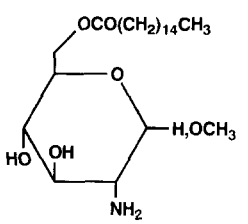

Fig. 1. Structure of methyl-2-amino-2-deoxy-6-*O*-palmitoyl-D-glucoside (PGlcN).

(dd, $J = 6.6$ Hz, $J = 6.8$ Hz, 1H), 4.30 (d, $J = 10.6$ Hz, 1H), 4.51 (d, $J = 3.4$ Hz, 1H), 5.00 (m, 1H), 5.15 (m, 1H) The CMC value of PGlcN was 0.71 μM using a TNS fluorescence technique (Nakagaki et al., 1986).

Other monoesters of neutral and basic sugars were synthesized in a similar manner.

2.3. Preparation of liposomes

Multilamellar vesicles (MLVs) were prepared by extrusion (Olson et al., 1979). Liposomes composed of egg phosphatidylcholine and cholesterol (8:2, molar ratio) were used as controls and those composed of egg phosphatidylcholine, cholesterol and glycolipid (6:2:2, molar ratio) as sugar coated liposomes. The lipid mixtures in chloroform were evaporated to form thin lipid films. To prepare lipid labeled liposomes, [^{3}H]-CHE as a non-exchangeable and non-degradable marker was added to the lipid mixture. The thin lipid film was dried overnight in vacuo, then hydrated with phosphate-buffered saline (8.1 mM Na_2HPO_4/ 1.47 mM KH_2PO_4/ 137.9 mM NaCl/ 2.7 mM KCl, PBS, pH 7.4). The suspensions were successively extruded through polycarbonate filters of various pore sizes (0.6 and 0.2 μm, five times; VET200, vesicles with a diameter of 200 nm prepared by extrusion technique). The sizes of the liposomes were measured by dynamic light scattering on a Photal laser particle analyzer (LPA-3100, Otsuka Electronics Co. Ltd, Japan) connected to a photon correlater (LPA-3000). The zeta potential of liposomes with a diameter of about 200 nm was calculated using the Smoluchowski equation (Adamson, 1967) from their electrophoretic mobilities in PBS (pH 7.4) obtained at 25°C, from a electrophoretic light scattering spectrophotometer (Zetasizer 4, Malvern Instruments, U.K.).

2.4. Hemolytic activities of positively charged liposomes

Stearylamine enhances the permeability of the biomembrane and damages the cells (Jonas and Speller, 1989; Yoshihara and Nakae, 1986). Therefore, the hemolytic activity of positively charged liposomes (incorporation of 20 mol% PGLN or SA) was investigated to compare SA and PGLNs. Liposomes were prepared by extrusion in 10 mM Hepes/150 mM NaCl (pH 7.4) buffer. Rat erythrocytes were dispersed in the same buffer, mixed with a liposomal suspension, then incubated at 37°C. After 1 h, the suspension was centrifuged and the absorbance of supernatants was measured at 540 nm on a spectrophotometer (UV-265FW, Shimadzu Co., Japan). The absorbance corresponding to 100% hemolysis was determined by adding 5 ml of water to 1 ml of the erythrocyte suspension. The lipid concentration was determined according to Bartlett (1959).

2.5. Toxicity of liposomes

Since SA liposomes show toxicity (Mayhew et al., 1987), the lethal dose of positively charged liposomes (incorporation of 20 mol% PGLN or SA) was investigated. Liposomes were injected into the tail vein of 10 male ddy mice weighing 25 g and the survival was observed immediately after the injection.

2.6. Stability of liposomes in serum

The thin lipid films were hydrated with 1 mM calcein/10 mM Tris/150 mM NaCl buffer (pH 7.4). The suspensions were vortexed and extruded through polycarbonate filters as described above. Unentrapped calcein was removed by gel filtration (Bio-Gel A-1.5m, 10 mM Tris/150 mM NaCl buffer (pH 7.4) as an elute). 1 ml of the liposomal suspension was mixed with 4 ml of prewarmed rat serum and the mixture was incubated at 37°C. The retention of calcein in liposomes was determined fluorometrically (excitation at 490 nm and emission at 520 nm) on a spectrofluorometer (RF-5000, Shimadzu Co., Japan). The percentage of retention was calculated from Eq. 1:

$$\text{retention}\ (\%) = \left(1 - \frac{F_1 - F_2 \times \frac{3.1}{3}}{F_3 \times \frac{3.1}{3}}\right) \times 100 \quad (1)$$

where F_2 and F_1 respectively are the fluorescence intensity of calcein entrapped in liposomes with or without 1 mM cobalt chloride (Sawahara et al., 1991). F_3 is the fluorescence intensity of calcein after addition of 10% v/v Triton X-100 corresponding to 100% leakage.

2.7. Studies in vivo

Liposomes labeled with [^{3}H]CHE (15 μmol of total lipid per kg) were injected into the femoral vein of three Nembutal-anesthetized male Wistar rats, weighing from 180 to 200 g. At regular intervals, blood samples were collected from the jugular vein with a heparinized syringe and centrifuged at $2000 \times g$ for 2 min to obtain the plasma. To determine the amounts of liposomes accumulated in each organ at 8 h after injection, the liver was perfused via the portal vein with isotonic saline to remove the blood. Thereafter, the liver, spleen, kidney, heart, lung, intestine, muscle and lymph were collected and washed with saline. Around 50 mg of tissue was dissolved in Soluene-350, neutralized with HCl, then Clearsol I was added. The radioactivity of samples was counted on a scintillation counter (LS5000TA, Beckman, U.S.A.). The tissue samples were examined in triplicate.

The plasma liposome concentrations vs time data after intravenous injection showed a biexponential decay profile. The time course of the concentration of lipid in plasma was therefore numerically fitted to a two-compartment model using MULTI (Yamaoka et al., 1981).

2.8. Lectin-induced aggregation

Momordica charantia lectin was added rapidly to a liposomal suspension, in 10 mM Tris/150 mM NaCl (pH 7.4) buffer, aggregation was followed by monitoring the turbidity increase with time at 540 nm on a spectrophotometer (UV-265FW, Shimadzu Co., Japan) at 25°C (Curatolo et al., 1978). The incubation mixtures contained liposomes at a total lipid concentration of 0.5 mM and 50 μg of lectin, in a total volume of 2.1 ml.

3. Results

3.1. Properties of liposomes

Table 1 shows the average diameter and zeta potential of the liposomes studied here. Based on dynamic light scattering, the mean diameters of liposomes were about 200 nm with homogeneous distribution. Electron micrographs revealed that the liposomes were multilamellar. The zeta potentials of control and PGL liposomes were about 0 mV. Those of PGLN liposomes were about 10 mV. These results indicated that the PGLNs were stably incorporated into the egg PC/cholesterol bilayer.

3.2. Hemolytic activities and toxicity of positively charged liposomes

SA liposomes showed hemolytic activity at high lipid concentration range (Fig. 2). However, there was no apparent hemolytic activity of PGlcN liposomes. PGalN and PManN liposomes also showed no hemolytic activity (data not shown).

The 50% lethal dose of SA liposomes was about 1.5 mmol total lipid/kg in mice, however, those of control liposomes and PGlcN liposomes could not be determined (Fig. 3). Those values would be much greater than that of SA liposomes.

3.3. Stability of liposomes in serum

Table 2 shows the calcein retention in rat serum at 37°C. About 90% of calcein was re-

Table 1
Physico-chemical properties of liposomes

Lipid composition (molar ratio)	Diameter (nm)	Zeta potential (mV)
EPC/Chol = 8:2	236.8 ± 33	−3.06 ± 0.66
EPC/Chol/PGlcN = 6:2:2	225.8 ± 25	10.52 ± 0.26
EPC/Chol/PGalN = 6:2:2	237.6 ± 44	12.46 ± 0.40
EPC/Chol/PManN = 6:2:2	243.8 ± 49	8.58 ± 0.44
EPC/Chol/PGlc = 6:2:2	197.5 ± 21	−2.60 ± 0.38
EPC/Chol/PGal = 6:2:2	217.1 ± 21	−3.10 ± 0.80
EPC/Chol/PMan = 6:2:2	238.7 ± 24	−2.44 ± 0.36
EPC/Chol/SA = 6:2:2	202.6 ± 44	23.06 ± 0.52

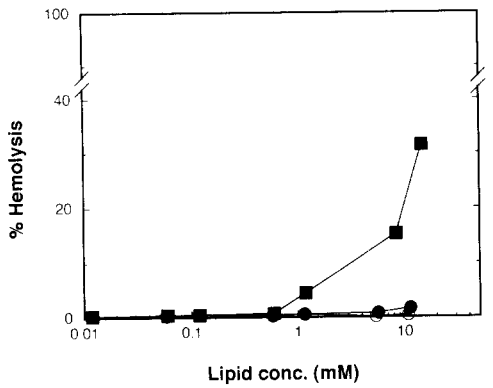

Fig. 2. Hemolytic activity of liposomes. The values are expressed as a percentage of hemolysis. (●) EPC/Chol (8:2 molar ratio); (○) EPC/Chol/PGlcN (6:2:2 molar ratio); (■) EPC/Chol/SA (6:2:2 molar ratio).

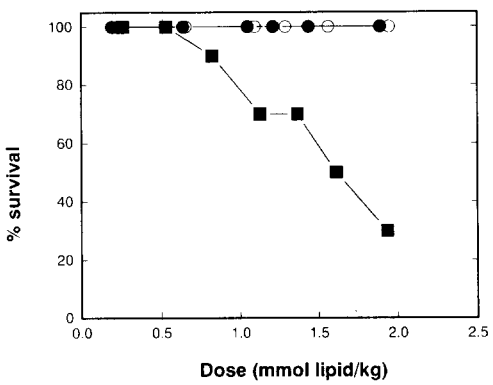

Fig. 3. Survival of mice treated with liposomes. (●) EPC/Chol (8:2 molar ratio); (○) EPC/Chol/PGlcN (6:2:2 molar ratio); (■) EPC/Chol/SA (6:2:2 molar ratio).

Table 2
Calcein retention of liposomes in rat serum for 8 h at 37°C

Lipid composition	Retention (%)
EPC/Chol = 8:2	90.2
EPC/Chol/PGlcN = 6:2:2	90.7
EPC/Chol/PGalN = 6:2:2	89.1
EPC/Chol/PManN = 6:2:2	89.3
EPC/Chol/PGlc = 6:2:2	88.1
EPC/Chol/PGal = 6:2:2	87.0
EPC/Chol/PMan = 6:2:2	87.4

The liposomes described above were prepared as described in the text. Calcein retention in liposomes was determined fluorometrically.

tained in all liposomes after an 8 h incubation with rat serum. Incorporation of PGLNs into the egg PC/cholesterol bilayer did not cause an increase in calcein leakage from the liposomes in rat serum. PGL liposomes also were stable in rat serum.

3.4. Studies in vivo

The radioactivity in plasma of PGLN liposomes was greater than that of controls in the plasma at every point determined (Fig. 4). The estimated AUC and MRT values of PGLN liposomes were 2-fold larger than those of controls (Table 3). The uptake of PGLN liposomes in the liver at 8 h after i.v. administration decreased compared with that of controls (Fig. 5). The radioactivity of SA liposomes in the plasma immediately after the injection was lower than that of controls, however, it then decreased more slowly, and became greater than that of controls after 8 h. Thus, V_{ss} and MRT of SA liposomes were larger than those of controls, and the AUC (or

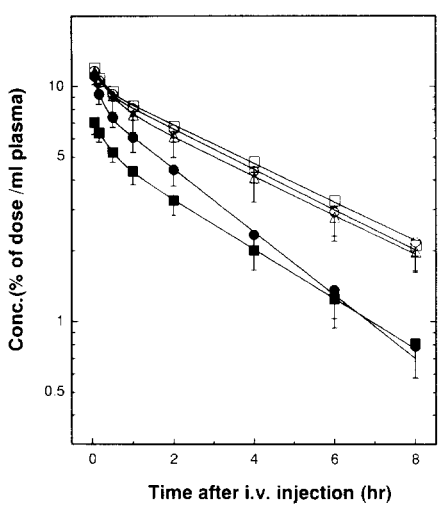

Fig. 4. Blood clearance of PGLN and SA liposomes in the rat after an intravenous injection. The liposomes were labeled with [^{3}H]CHE. Each value is expressed as a percentage ± S.D. of the administered [^{3}H]CHE radioactivity per ml of plasma ($n = 3$). (●) EPC/Chol (8:2 molar ratio); (○) EPC/Chol/PGlcN (6:2:2 molar ratio); (□) EPC/Chol/PGalN (6:2:2 molar ratio); (△) EPC/Chol/PManN (6:2:2 molar ratio); (■) EPC/Chol/SA (6:2:2 molar ratio).

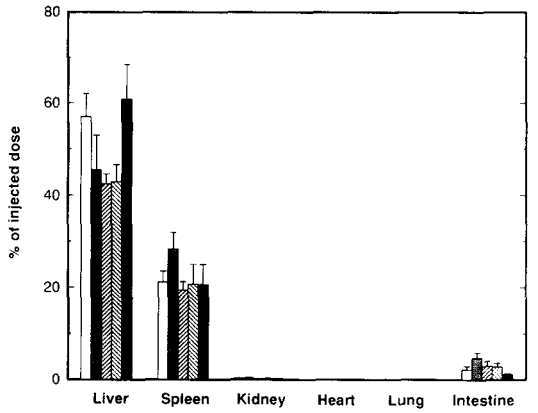

Fig. 5. Tissue distribution of PGLN liposomes in the rat 8 h after an intravenous injection. The liposomes were labeled with [^{3}H]CHE. Each value is expressed as a percentage ± S.D. of the administered [^{3}H]CHE radioactivity per total organ ($n = 3$). (□) EPC/Chol (8:2 molar ratio); (▩) EPC/Chol/PGlcN (6:2:2 molar ratio); (▨) EPC/Chol/PGalN (6:2:2 molar ratio); (▧) EPC/Chol/PManN (6:2:2 molar ratio); (□) EPC/Chol/SA (6:2:2 molar ratio).

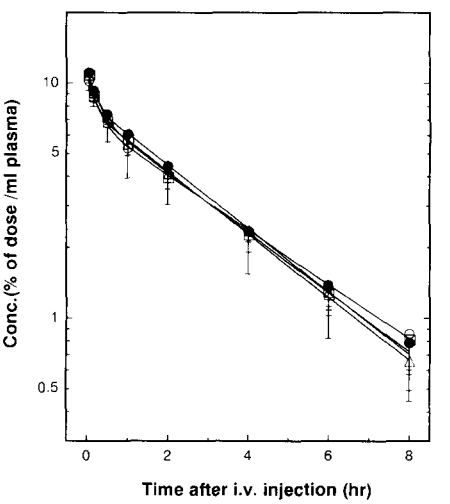

Fig. 6. Blood clearance of PGL liposomes in the rat after an intravenous injection. The liposomes were labeled with [^{3}H] CHE Each value is expressed as a percentage ± S.D. of the administered [^{3}H]CHE radioactivity per ml of plasma ($n = 3$). (●) EPC/Chol (8:2 molar ratio); (○) EPC/Chol/PGlc (6:2:2 molar ratio); (□) EPC/Chol/PGal (6:2:2 molar ratio); (△) EPC/Chol/PMan (6:2:2 molar ratio).

CL) of SA liposomes was almost the same as that of controls (Table 3). In contrast to the positively charged PGLN liposomes, the disposition characteristics of the PGL liposomes were almost identical to that of controls (Fig. 6). The tissue distribution of PGL liposomes was not different from those of the controls 8 h after i.v. injection (data not shown).

3.5. Lectin-induced aggregation

The increase in turbidity was observed by the addition of *M. charantia* lectin to a suspension of LacCer liposomes (incorporation of 20 mol% lactosylceramide), while no aggregation was ob-

Table 3
The pharmacokinetic parameters of liposomes following intravenous injection into the rat

Lipid composition	AUC (% dose h ml^{-1})	MRT (h)	CL (ml h^{-1} kg^{-1})	V_{SS} (ml)
EPC/Chol = 8:2	27.5 ± 4.6	3.1 ± 0.3	17.8 ± 2.5	11.6 ± 0.9
EPC/Chol/PGlcN = 6:2:2	52.2 ± 10.2 [b]	5.3 ± 1.0 [b]	9.4 ± 1.6 [b]	10.2 ± 0.9
EPC/Chol/PGalN = 6:2:2	52.6 ± 5.8 [b]	5.1 ± 0.3 [b]	9.5 ± 1.1 [b]	9.8 ± 0.9
EPC/Chol/PManN = 6:2:2	48.2 ± 9.4 [b]	5.1 ± 0.3 [b]	9.8 ± 1.8 [b]	10.8 ± 2.0
EPC/Chol/SA = 6:2:2	23.5 ± 4.1	4.3 ± 0.9 [a]	20.4 ± 4.2	18.5 ± 2.5 [b]
EPC/Chol/PGlc = 6:2:2	27.2 ± 7.8	3.7 ± 0.6	18.5 ± 5.8	14.5 ± 4.3
EPC/Chol/PGal = 6:2:2	26.6 ± 2.6	3.3 ± 0.3	19.3 ± 2.1	12.2 ± 0.2
EPC/Chol/PMan = 6:2:2	26.4 ± 2.8	3.3 ± 0.0	18.9 ± 1.4	12.7 ± 1.2

Liposomes (15 μmol of total lipid per kg) were injected intravenously into male Wistar rats. The values were obtained from fitting program MULTI and are expressed as means ± S.D..
[a] Significant difference from control liposomes ($0.01 < p < 0.05$).
[b] Significant difference from control liposomes ($p < 0.01$).

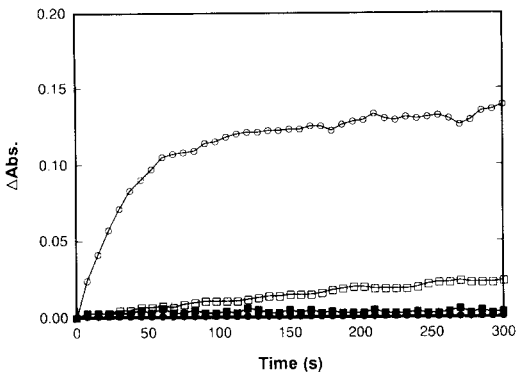

Fig. 7. Lectin-induced aggregation as followed by turbidity. (●) EPC/Chol (8:2 molar ratio); (■) EPC/Chol/PGalN (6:2:2 molar ratio); (□) EPC/Chol/PGal (6:2:2 molar ratio); (○) EPC/Chol/LacCer (6:2:2 molar ratio).

served during the same treatment in the case of PGalN and PGal liposomes (Fig. 7).

4. Discussion

Positively charged liposomes containing PGLN had a longer half-life in the bloodstream than controls and accumulated to a lesser extent in liver after intravenous injection. It is predicted that the suppression of the hepatic uptake of these liposomes retards the residence time. The spillover phenomenon may be the cause of the lack of decrease in liposomal uptake in the spleen (Dave and Patel, 1986). The disposition kinetics of PGL liposomes, however, were almost the same as that of controls. Allen et al. (1989) showed that glucosylceramide, monoglucosyldiacylglycerol and galactosylceramide were also incapable of decreasing the uptake of liposomes by RES. These investigations suggest the positive charge on PGLNs may be one important factor avoiding RES.

Small liposomes of diameters below 0.1 μm can pass through the fenestration in the sinusoids or through the region of increased capillary permeability and gain access to liver parenchymal cells (Poste et al., 1982). Therefore, most of the liver uptake of multilamellar vesicles with a diameter of 0.2 μm may be carried out by the Kupffer cells. The parenchymal cells have a galactose specific receptor and the nonparenchymal cells have a mannose one (Ghosh and Bachhawat, 1980). Consequently, liposomes containing lactosylceramide effectively accumulate in the liver (Spanjer and Scherphof, 1983; Spanjer et al., 1984). PGalN, however, decreased the hepatic uptake of liposomes and PGal did not affect the uptake. Allen et al. (1989) reported that galactosylceramide does not also enhance the liver uptake of liposomes. Lectin-induced aggregation indicates that the galactose residue in PGalN or PGal is not positioned above the phospholipid choline groups and lectin is inaccessible to this residue. That is to say, when the sugar residue in PGLNs or PGLs is not exposed on the liposomal surface, the liver cells cannot recognize it, suggesting that the positive charge plays an important role in the fate of liposome. Disaccharides, polysaccharides or glycolipids with some spacers between sugar moiety and the hydrophobic group seem to be a necessary for effective delivery of liposomes to the liver.

The hemolytic activity of PGLN liposomes is lower than that of SA liposomes and our preliminary studies showed that the lethal dose of PGLN liposomes is much higher than that of SA liposomes in mice. SA liposomes act on the acidic phospholipid and cause membrane damage (Yoshihara and Nakae, 1986). No hemolytic activity of PGLN liposomes suggests that the interactions with erythrocytes are weaker than or different from that of SA liposomes. The different behavior in vivo between PGLN liposomes and SA liposomes may be attributable to the interactions with erythrocytes. One explanation for this effect is the hydrophilicity of the hydroxy group of the sugar moiety. The difference in the stability of SA liposomes in blood circulation between ours and those of Gregoriadis and Neerunjun (1974) may be expained by the assay method of the samples. The latter authors determined the liposomal concentration in total blood, while we determined those in plasma.

Nicholas and Jones (1991) showed by rat liver perfusion that dipalmitoylphosphatidylcholine (DPPC)/SA 10% w/w liposomes bound to the erythrocytes and suppressed liver uptake in the

presence of blood. The binding of SA liposomes to the erythrocytes may lead to the initially low radioactivity in the plasma. In a preliminary experiment, considerable accumulation of PGlcN liposomes was observed from the recirculating perfusion experiment with Hanks buffer as a perfusate using rat liver. However, the accumulation in the liver was suppressed when rat red blood cells were added to the perfusate. This fact seems to suggest that the blood cells play an important role for PGLN liposomes to avoid RES.

Namba et al. (1990) reported that modification using negatively charged sugar glucuronate (PGlcUA) led to a prolonged residence time of liposomes. The mechanism via which PGlcUA liposomes avoid the RES is not clear. However, the mechanism would be different from that of our liposomes modified by positively charged sugar.

In conclusion, PGLNs can decrease the uptake of liposomes by the liver and prolong the lifetime in the blood circulation. The effect of PGLN incorporation into liposomes of reducing their uptake by the liver depends on the positive charge and the lack of a spacer may result in PGLN or PGL liposomes escaping cellular recognition. However, the mechanisms of avoiding RES by PGLNs need further consideration.

PGLN liposomes provide several advantages. Besides the long half-life in the blood circulation, PGLN liposomes can entrap a large amount of negatively charged substances through electrostatic interactions, as compared with neutral liposomes (Miyajima et al., 1993). The amino sugar coated liposomes have several advantages as mentioned above and show promise for therapeutic applications.

Acknowledgments

This work was supported in part by a Grant-in-Aid (No. 06772103) for Encouragement of Young Scientists from the Ministry of Education, Science and Culture of Japan. We thank Dr K. Nishida for technical assistance, and Dr K. Yamaoka for helpful discussion.

References

Adams, D.H., Joyce, G., Richardson, V.J., Ryman, B.E. and Wisniewski, H.M., Liposome toxicity in the mouse central nervous system. *J. Neur. Sci.*, 31 (1977) 173–179.

Adamson, A.W., *Physical Chemistry of Surface*, 2nd Edn, Interscience, New York, 1967, ch. 9, p. 223.

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. and Rutledge, J., Liposomes with prolonged circulation times: factors affecting uptake by reticuloendothelial and other tissues. *Biochim. Biophys. Acta*, 981 (1989) 27–35.

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.

Alving, C.R., Steck, E.A., Chapman, W.L. Jr, Waits, V.B., Hendricks, L.D., Swartz, G.M. Jr and Hanson, W.L., Therapy of leishmaniasis: Superior efficacies of liposome-encapsulated drugs. *Proc. Natl. Acad. Sci. USA*, 75 (1978) 2959–2963.

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

Blume, G. and Cevc, G., Molecular mechanism of the lipid vesicle longevity in vivo. *Biochim. Biophys. Acta*, 1146 (1993) 157–168.

Chance, M.L., Thomas, S.C. and Peters, W., Antileishmanial activity of antimonials entrapped in liposomes. *Nature*, 272 (1978) 55–56.

Curatolo, W., Yau, A.O., Small, D.M. and Sears, B., Lectin-induced agglutination of phospholipid/glycolipid vesicles. *Biochemistry*, 17 (1978) 5740–5744.

Dave, J. and Patel, H.M., Differentiation hepatic and splenic phagocytic activity during reticuloendothelial blockade with cholesterol-free and cholesterol-rich liposomes. *Biochim. Biophys. Acta*, 888 (1986) 184–190.

Ghosh, P. and Bachhawat, B.K., Grafting of different glycosides on the surface of liposomes and its effect on the tissue distribution of 125I-labelled g-globulin encapsulated in liposomes. *Biochim. Biophys. Acta*, 632 (1980) 562–572.

Gregoriadis, G., *Liposomes as Drug Carriers*, ch. 1, Wiley, Chichester, 1988.

Gregoriadis, G. and Allison, A.C., *Liposomes in Biological Systems*, Wiley, Chichester, 1980.

Gregoriadis, G. and Neerunjun, D.E., Control of the rate of hepatic uptake and catabolism of liposome-entrapped proteins injected into rats. Possible therapeutic applications. *Eur. J. Biochem.*, 47 (1974) 179–185.

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.

Jonas, A.J. and Speller, R.J., Stearylamine permeabilizes the lysosomal membrane to cystine and sialic acid. *Biochim. Biophys. Acta*, 984 (1989) 257–261.

Mayhew, E., Ito, M. and Lazo, R., Toxicity of non-drug-containing liposomes for cultured human cells. *Exp. Cell Res.*, 171 (1987) 195–202.

Miyajima, K., Komatsu, H., Sun, C., Aoki, H., Handa, T., Xu, H., Fuji, K. and Okada, S., Effects of cholesterol on the miscibility of synthetic glucosamine diesters in lipid bilayers and the entrapment of superoxide dismutase into the positively charged liposmes. *Chem. Pharm. Bull.*, 41 (1993) 1889–1894.

Nakagaki, M., Komatsu, H. and Handa, T., Estimation of critical micelle concentrations of lysolecithins with fluorescent probes. *Chem. Pharm. Bull.*, 34 (1986) 4479–4485.

Namba, Y., Sakakibara, T., Masuda, M., Ito, F. and Oku, N., Glucuronate-modified liposomes with Prolonged circulation time. *Chem. Pharm. Bull.*, 38 (1990) 1663–1666.

Nicholas, A.R. and Jones, M.N., The effect of blood on the uptake of liposomal lipid by perfused rat liver. *Biochim. Biophys. Acta*, 1074 (1991) 105–111.

Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J. and Papahadjopoulos, D., Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. *Biochim. Biophys. Acta*, 557 (1979) 9–23.

Poste, G., Bucana, C., Raz, A., Bugelski, P., Kirsh, R. and Fidler, I.J., Analysis of the fate of systemically administered liposomes and implications for their use in drug delivery. *Cancer Res.*, 42 (1982) 1412–1422.

Sawahara, H., Goto, S. and Kinoshita, N., Double fluorescent labeling method used for a study on liposomes. *Chem. Pharm. Bull.*, 39 (1991) 227–229.

Senior, J. and Gregoriadis, G., Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of phospholipid and cholesterol components. *Life Sci.*, 30 (1982) 2123–2136.

Senior, J., Crawley, J.C.W. and Greogoriadis, G., Tissue distribution of liposomes exhibiting long half-lives in the circulation after intravenous injection. *Biochim. Biophys. Acta*, 839 (1985) 1–8.

Spanjer, H.H. and Scherphof, G.L., Targeting of lactosylceramide-containing liposomes to hepatocytes in vivo. *Biochim. Biophys. Acta*, 734 (1983) 40–47.

Spanjer, H.H., Morselt, H. and Scherphof, G.L., Lactosylceramide-induce stimulation of liposome uptake by Kupffer cells in vivo. *Biochim. Biophys. Acta*, 774 (1984) 49–55.

Woodle, M.C., Matthay, K.K., Newman, M.S., Hidayat, J.E., Collins, L.R., Redemann, C., Martin, F.J. and Papahadjopoulos, D., Versatility in lipid compositions showing prolonged circulation with sterically sabilized liposomes. *Biochim. Biophys. Acta*, 1105 (1992) 193–200.

Yamaoka, K., Tanigawara, Y. and Nakagawa, T., A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharm. Dyn.*, 4 (1981) 879–890.

Yoshihara, E. and Nakae, T., Cytolytic activity of liposomes containing stearylamine. *Biochim. Biophys. Acta*, 854 (1986) 93–101.