Novel Long-circulating Liposomes Containing Peptide Library-lipid Conjugates: Synthesis and In Vivo Behavior

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Rapid uptake of intravenously injected liposomes by the mononuclear phagocyte system has limited their use as drug delivery vehicles. Recently, various long-circulating liposomes have been prepared by incorporating glycolipids or other amphiphilic molecules into the lipid bilayer of conventional liposomes. The purpose of the present study was to design a new class of biodegradable membrane modifiers that would increase the half-life of liposomes in vivo. Using solid-phase peptide synthesis, synthesized were 30-residue random libraries consisting of a random sequence of glycine, β-alanine and γ-aminobutyric acid. The libraries were coupled to stearic acid (SA) or phosphatidylethanolamine (PE). The resulting amphiphilic conjugates were mixed with egg phosphatidylcholine (PC) and cholesterol (Chol) in a 6:47:47 ratio, and unilamellar liposomes were prepared. For comparison, plain PC/Chol (50:50) liposomes, as well as liposomes containing polyethylene glycol (PEG)-SA/PC/Chol (6:47:47) and PEG-PE/PC/Chol (6:47:47) were also prepared. Calcein was entrapped in the liposomes, which were given intravenously to rats at a dose of 9.2 μmol/lipid/kg, and the amount of intact liposomes present in serum was followed with time. While the conventional liposomes had a short elimination half-life (28 min), the liposomes modified with library-PE had a much longer half-life (170 min), while library-SA provided no improvement of the liposome pharmacokinetics. PEG-PE greatly improved the half-life of the liposomes (400 min) while PEG-SA only provided a marginal improvement. All liposome preparations were cleared in a biphasic fashion.

In conclusion, a novel biodegradable lipopeptide conjugate was designed that endows liposomes with a prolonged circulation time in vivo. The pharmacokinetic profile of these modified liposomes was drastically improved over that of conventional liposomes. Since the library is prepared by solid-phase synthesis, length and/or composition could easily be modified in order to modulate the clearance profile of the liposomes. Tailoring of the pharmacokinetic profile of the liposomes depending on their intended application may allow for a greater flexibility of use than PEG-PE.

Keywords: Liposomes; Biodegradable; Peptides; Amino acid; PEG

INTRODUCTION

Liposomes can be versatile intravascular drug delivery systems, but they are easily recognized by the mononuclear phagocyte system and are cleared from the circulation in a dose-dependent manner. Many strategies have been developed to improve liposome pharmacokinetic profile, and surface modification with polyethylene glycol (PEG) has been the most successful method to date. Incorporation of a PEG-lipid conjugate into liposome bilayers appears to delay liposome clearance by providing steric hindrance, thereby shielding the liposomes from opsonin binding and delaying uptake by liver macrophages (Zeisig et al., 1996). A notable benefit of long-circulating liposomes is that they accumulate passively in pathological sites with increased vascular permeability such as tumors or inflamed tissues without any need for specific targeting (Gabizon, 2002). Pegylated, so-called “Stealth” liposomes containing doxorubicin are currently used in patients suffering from Kaposi’s sarcoma, resistant ovarian cancer or metastatic breast cancer. They have many advantages compared to the free drug, such as a longer circulation half-life, a lower toxicity and specific accumulation in tumor tissues (Lian and Ho, 2001).

Low molecular-weight PEG is not toxic, but it is not biodegradable, and long-term effects of treatment with PEG-liposomes are unknown (Matthews et al., 1996). The goal of the present study was to generate sterically stabilized liposomes with properties similar to those of PEG-stabilized liposomes using a biodegradable macromolecule-lipid conjugate as liposome modifier. It has been shown that liposome modifiers should be water-soluble, hydrophilic and flexible, to result in a large number of probable conformations, or “cloud”, providing steric
been developed (Torchilin et al., 2001). A peptide library was designed to have sites prone to hydrogen bonding, be biodegradable and water-soluble. The planar property of amide bonds limits the flexibility of peptides; however, the use of unnatural amino acids having more than one carbon between two successive amide bonds provides flexibility to the peptide. Three amino acids were used: glycine and β-alanine, and γ-aminobutyric acid. The flexibility of the peptide was maximized by generating a combinatorial library of the three amino acids, thereby preventing the possible formation of a secondary structure not only within a strand but also between adjacent strands.

Since antibodies are usually targeted towards specific side chains such as aromatic moieties in a rigid peptide, a peptide containing only amino acids without any side-chain is expected to be minimally antigenic. It is also anticipated that a peptide of β-alanine and γ-aminobutyric acid would be biochemically stable enough within the vasculature bed for prolonging the circulatory life of liposomes and yet ultimately degraded by a host of peptidases. In addition, the macromolecule was designed to have a size comparable to that of PEG 1900, which gives the best results in vivo when bound to PE and incorporated into liposomes (Allen, 1994). The average molecular weight per residue being 85, a 30-residue peptide was prepared. The library was expected to contain a mixture of the 3⁶⁰ possible peptide sequences, and to be about 2.5 kDa in molecular weight. It was then conjugated to a lipid, incorporated into liposomes, and the pharmacokinetics of the resulting liposomes were measured in rats subsequent to intravenous bolus injection.

MATERIALS AND METHODS

Materials

The following compounds were purchased from Sigma Chemicals (St Louis, MO) and used as received: O-benzotriazole-N,N,N,N′-tetramethyl uronium hexafluorophosphate (HBTU), trifluoroacetic acid (TFA), N-hydroxybenzotriazole (HOBt), N-methyl morpholine (NMM), N,N-diisopropylethylamine (DIEA), stearic acid (SA), piperidine, cholesterol (Chol), N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES), Triton X-100, polyoxyethylene 40 stearate (Myrij 52), ascorbic acid and calcine. Ammonium molybdate (4%) was purchased from LabChem (Pittsburg, PA), Ethylenedinitrilotetraacetic acid (EDTA) was obtained from Mallinckrodt. Fluorenylmethoxycarbonyl-protected amino acids: N-α-Fmoc-glycine (Fmoc-Gly), N-β-Fmoc-β-alanine (Fmoc-Ala) and N-γ-Fmoc-γ-aminobutyric acid (Fmoc-GABA), as well as 4-(2′,4′-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl methylbenz-hydrylamine resin (Rink Amide MBHA resin) were purchased from Advanced ChemTech (Louisville, KY). The N-α-Fmoc-N-e-glycine-2-chlorotrityl resin was purchased from Solid Phase Science (San Rafael, CA). Egg phosphatidylcholine (PC), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG-PE), and N-succinyl-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (succinyl-PE) were purchased from Avanti Polar Lipids (Alabaster, AL) and used as received. All solvents used were of the highest purity available, and were stored over activated 4 Å molecular sieves and under argon.

Synthesis of Library-stearic Acid (Library-SA)

A 30-residue library was assembled on a 0.25 mmol scale from a mixture of three Fmoc-protected amino acids and Rink Amide MBHA resin by stepwise solid-phase synthesis using an Applied Biosystems Model 430A automated peptide synthesizer. A 4-fold excess of the Fmoc-amino acid mixture relative to the resin was used at each step. The ratio of (Fmoc-Gly/Fmoc-Ala/Fmoc-GABA) was (2:1:1). The activating agent HBTU was used in a 1:1 ratio with the total amount of amino acid, and N-methyl morpholine (NMM) was used in a 4:1 ratio with the total amount of amino acid. After synthesis, 25% of the resin was isolated, and subjected to cleavage by treatment with 10 ml of 95% TFA for 2 h. The peptide was precipitated with ether, and extracted with 40 ml water (2 times) followed by 40 ml of 10% acetic acid (2 times) and 40 ml of water (2 times). The peptide was lyophilized, neutralized with ammonium bicarbonate and lyophilized again. The remainder of the resin was used to synthesize library-SA. Stearic acid was reacted with the resin-bound library as if it were an amino acid, and was added in a 4-fold excess. The conjugate was cleaved from the resin and separated as described previously.

Synthesis of Library-phosphatidylethanolamine (Library-PE)

The 30-residue peptide library was assembled on a 25 μmol scale from a mixture of three Fmoc-protected amino acids and N-α-Fmoc-N-e-Bocglycine-2-chlorotrityl resin by stepwise solid-phase synthesis with a Rainin Symphony/Multiplex® automated peptide synthesizer. A 5-fold excess of the Fmoc-amino acid mixture relative to the resin was used at each step, and double coupling was used (two repetitions of the 20 min coupling per step). The ratio of (Fmoc-Gly/Fmoc-βAla/Fmoc-GABA) used in the synthesis was (2:1:1). The activating agent HBTU was used in a 1:1 ratio with the total amount of amino acid, and NMM was used in a 4:1 ratio with the amino acid.

Once the 29 steps were completed, the resin was thoroughly dried and weighted. Ten percent of the library-bound resin was deprotected then cleaved using 45% TFA in methylene chloride for 15 min. After precipitation with ether followed by three additional washings, the resulting peptide was freeze-dried. Half of the remaining resin (90 mg, 20 μmol) was manually coupled with succiny1-PE. The Fmoc group was removed with 20% piperidine in DMF for 30 min under argon atmosphere, rinsed and dried.
The solvent was evaporated under reduced pressure at room temperature. Egg phosphatidylcholine/cholesterol (PC/Chol) liposomes were prepared by mixing equal amounts of egg PC (23.5 μmoles) and cholesterol (23.5 μmoles) in chloroform. The solvent was evaporated under reduced pressure at room temperature. The resulting thin lipid film was dried further under high vacuum in a conventional lyophilizer overnight. A 70 mM calcine solution was prepared and titrated to pH 7.4 with sodium hydroxide. The osmolality was measured using a Fiske ONE-TEN™ Osmometer (Fiske Associates, Norwood, MA) and adjusted to that of blood (290 ± 5 mOsm/kg) with sodium chloride. One milliliter of this solution was added to the lipid film, which was hydrated for 30 min. The mixture was sonicated and freeze-thawed 10 times between dry ice/acetone and a water bath at room temperature. The resulting multimellar vesicles were allowed to anneal by gentle rotation at 40°C for 1 h.

The above preparation was then extruded 10 times under nitrogen pressure through two stacked 0.2-μm polycarbonate membrane filters (Osmonics, Livermore, CA) held by a jacketed extrusion cell (Lipex Bio- membranes Inc., Vancouver, BC, Canada). This process is known to result in the formation of large unilamellar vesicles. Unentrapped calcine was separated from the liposomes by size-exclusion chromatography using spin-columns containing Sepharose CL-6B gel (Pharmacia LKB, Piscataway, NJ), and 50 mM HEPES buffer (pH 7.4, made isotonic with blood using sodium chloride) was used as the eluent. The assembly was spun at 300 g for 30 s for each milliliter of the eluent added successively. The resulting liposome solution was brought to a volume of 10 ml with the buffer. The PC/Chol/PEG-SA, PC/Chol/library-SA, PC/Chol/PEG-PE and PC/Chol/library-PE liposomes were prepared by the same method as described above for the PC/Chol liposomes, with PEG-SA (Myrj 52, MW 2000, 3 μmoles), library-SA (3 μmoles), PEG-PE (MW 2000, 3 μmoles), library-PE, respectively mixed with the calcine solution before it was added to the lipid film. The resulting liposomes had a surface modifier/PC/Chol ratio of (6:47:47). A water bath temperature of 60°C was used for the liposomes containing PE.

**Characterization of Modifiers**

Library samples were degraded by gas-phase hydrolysis, derivatized and amino acid analysis was performed using standard methods. Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) was performed using a Kratos MALDI III instrument. The liposome modifier samples were diluted in a 50% acetonitrile solution containing 0.1% TFA. The matrix was 10 mg/ml α-cyano-4-hydroxycinnamic acid in 70% acetonitrile/30% of 0.1% TFA. Positive-ion electrospray ionization (ESI) mass spectra of the liposome modifiers were obtained on a Micromass Quattro II Electrospray Triple Quadrupole mass spectrometer. Samples were dissolved in a mixture of water, acetonitrile and acetic acid (69:30:1) to obtain a 10 μl/ml concentration, and were infused at a rate of 10 μl/min.

**Liposome Preparation**

Egg phosphatidylcholine/cholesterol (PC/Chol) liposomes were prepared by mixing equal amounts of egg PC (23.5 μmoles) and cholesterol (23.5 μmoles) in chloroform. The solvent was evaporated under reduced pressure at room temperature. The resulting thin lipid film was dried further under high vacuum in a conventional lyophilizer overnight. A 70 mM calcine solution was prepared and titrated to pH 7.4 with sodium hydroxide. The osmolality was measured using a Fiske ONE-TEN™ Osmometer (Fiske Associates, Norwood, MA) and adjusted to that of blood (290 ± 5 mOsm/kg) with sodium chloride. One milliliter of this solution was added to the lipid film, which was hydrated for 30 min. The mixture was sonicated and freeze-thawed 10 times between dry ice/acetone and a water bath at room temperature. The resulting multimellar vesicles were allowed to anneal by gentle rotation at 40°C for 1 h.

The above preparation was then extruded 10 times under nitrogen pressure through two stacked 0.2-μm polycarbonate membrane filters (Osmonics, Livermore, CA) held by a jacketed extrusion cell (Lipex Bio- membranes Inc., Vancouver, BC, Canada). This process is known to result in the formation of large unilamellar vesicles. Unentrapped calcine was separated from the liposomes by size-exclusion chromatography using spin-columns containing Sepharose CL-6B gel (Pharmacia LKB, Piscataway, NJ), and 50 mM HEPES buffer (pH 7.4, made isotonic with blood using sodium chloride) was used as the eluent. The assembly was spun at 300 g for 30 s for each milliliter of the eluent added successively. The resulting liposome solution was brought to a volume of 10 ml with the buffer. The PC/Chol/PEG-SA, PC/Chol/library-SA, PC/Chol/PEG-PE and PC/Chol/library-PE liposomes were prepared by the same method as described above for the PC/Chol liposomes, with PEG-SA (Myrj 52, MW 2000, 3 μmoles), library-SA (3 μmoles), PEG-PE (MW 2000, 3 μmoles), library-PE, respectively mixed with the calcine solution before it was added to the lipid film. The resulting liposomes had a surface modifier/PC/Chol ratio of (6:47:47). A water bath temperature of 60°C was used for the liposomes containing PE.

**Liposome Characterization**

Liposome size was measured by dynamic laser light scattering (Nicomp Model 370 Particle Sizing Systems, Santa Barbara, CA). The amount of lipid present in each sample was measured using the assay developed by Stewart (1980). The amount of calcine entrapped in the liposomes was measured by fluorescence (Ex = 487 nm and Em = 515 nm). Unentrapped calcine was separated from the samples immediately before the animal experiments using a spin column containing Sepharose CL-6B size-exclusion gel.

**Animal Experiments**

All animal procedures were in accordance with animal welfare guidelines and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats were purchased from Charles River (Raleigh, NC). They were allowed to get acclimated to the facility for at least 1 week prior to surgery. They had free access to food and water, and were on a 12 h-light/12 h-dark cycle in a room with controlled temperature and humidity. Rat weights ranged between 250 and 350 g at the time of the experiments. Five animals were randomly assigned to each of
the experimental groups. One day prior to each experiment, rats were anesthetized with ether and the left jugular vein was cannulated. The following day, each animal received intravenously 4.6 μmol of egg PC per kg of body weight, in a final volume of 1 ml with normal saline. The animals were awake and unrestrained during blood sampling.

Blood samples were collected through the cannula using a 3-way stopcock prior to administration and at given time intervals thereafter. Two hundred microliters of blood were collected at each time point, and were immediately replaced with an identical volume of normal saline. After centrifugation of the serum, two equal aliquots were taken from each sample. To the first one were added 500 μl of EDTA solution (66 mM) and enough isotonic HEPES buffer (50 mM, pH 7.4) to a final volume of 3 ml. The emission at 515 nm, representing the free calcein present in solution, was measured. To the second aliquot were added 500 μl of EDTA solution, 300 μl of Triton X-100 solution (2%, containing 0.02% sodium azide) and enough isotonic HEPES buffer to a final volume of 3 ml. The emission at 515 nm was measured. The difference between the emission intensities obtained from these two aliquots was a measure of the amount of calcein entrapped in the liposomes.

Sampling and Data Analysis

Serum concentration-time profiles were analyzed by fitting a mono or bi-exponential equation to the data with nonlinear least-squares regression to obtain estimates of non-compartmental pharmacokinetic parameters. These were calculated following standard procedures (Gibaldi and Perrier, 1982). All results were expressed as mean ± SD.

RESULTS AND DISCUSSION

A 30-residue peptide random library using three different amino acids was designed and coupled to stearic acid or phosphatidylethanolamine to serve as liposome membrane surface modifiers. The structures of the library and the various modifiers are shown in Fig. 1. The library was synthesized using Fmoc solid-phase peptide synthesis, and the lipid was attached during the last coupling step. A representative synthesis scheme is shown in Fig. 2. Linking a highly hydrophobic molecule to a hydrophilic peptide using solid phase chemistry revealed to be challenging, therefore, the last conjugation was performed manually for PE, and a large excess of the reagents was used in all cases. The library compounds were mixtures by design, and their molecular weight was in the range of 2–3 kDa. Thin-layer chromatography as well as phospholipid assays provided evidence that the desired products had been prepared (data not shown). Mass spectrometry was used to show the presence of a properly distributed library mixture for each of the compounds. MALDI mass spectrometry of the library synthesized on the Rink

![Figure 1](image-url) Structures of the four liposome modifiers used: (A) Library-SA (n = 1, 2 or 3); (B) PEG-SA; (C) library-PE (n = 1, 2 or 3); and (D) PEG-PE.
NOVEL LONG-CIRCULATING LIPOSOMES

**TABLE I** Diameter of the various liposome formulations after extrusion through polycarbonate filters (200 nm pore size), as measured by dynamic light scattering

<table>
<thead>
<tr>
<th>Liposome formulation</th>
<th>Diameter (nm) ± SD</th>
</tr>
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<tbody>
<tr>
<td>PC/Chol</td>
<td>198 ± 46</td>
</tr>
<tr>
<td>PC/Chol/PEG-SA</td>
<td>158 ± 39</td>
</tr>
<tr>
<td>PC/Chol/PEG-PE</td>
<td>167 ± 43</td>
</tr>
<tr>
<td>PC/Chol/Library-SA</td>
<td>170 ± 42</td>
</tr>
<tr>
<td>PC/Chol/Gly-Library-PE</td>
<td>174 ± 67</td>
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**FIGURE 2** Schematic representation of the synthesis of polyamide library-lipid conjugates.

Amide MBHA resin showed that the mixture was polydisperse, and that the average molecular weight was 2.2 kDa. The final product, library-SA, showed an equally polydisperse mixture with an average molecular weight of 2440 Da. This shift in molecular weight is consistent with the addition of stearic acid (MW 284). Amino acid analysis of the library synthesized on the chlorotrityl resin showed a ratio of 1:0.55:0.52 for Gly, βAla and GABA, respectively. MALDI mass spectrometry of the library showed a multiplet at 2255 Da. While MALDI mass spectrometry is a method of choice for large peptides, it was unsuccessful in determining the average molecular weight of the PE conjugates: the peaks obtained only reflected the presence of decomposition products. ESI mass spectrometry of library-PE showed a multiplet at 777 Da, which corresponds to M^+ and indicates an average molecular weight of 3.1 kDa.

For each of the various liposome formulations, 6 mol % of modifier was added to the PC/Chol liposomes, as it has been shown to be the optimum concentration for PEG-modified liposomes (Allen et al., 1991). The PE conjugates were expected to form micelles in water at low concentrations. The critical micelle concentration of DSPE-PEG 1900, for instance, is estimated to be less than 10^{-5} M and it forms micelles with an apparent hydrodynamic diameter of 20 nm (Woodle et al., 1992). If these micelles were present in equilibrium with liposomes in a given preparation, they were small enough to be separated from the liposomes along with unentrapped calcein when size-exclusion chromatography was performed. It was expected that the liposome fractions would be free of unentrapped compounds.

There is no consensus as to whether the protective effect exhibited by PEG comes from the steric barrier it creates on the liposome surface or from the fixed aqueous layer it creates around the liposomes, however, it is believed that the thickness of the fixed aqueous layer is one of the most important factors that determines the liposome clearance from the circulation. Table I shows the diameters of the liposomes obtained in the present study. All of the sterically modified liposomes appeared to be smaller than the conventional liposomes. Others have used various methods to measure the thickness of the layer surrounding PEG-grafted liposomes, and all indicate a thickness of 20–50 nm (Needham et al., 1992; Shimada et al., 1995) when PEG 2000 is used. The difference in size between PC/Chol liposomes and liposomes containing PEG-SA or PEG-PE is 40 and 31 nm, respectively, which is in accordance with the published measurements. It appears that the four liposome formulations containing the liposome modifiers have similar sizes. This suggests that the protective layer provided by the library is of the same order of magnitude as that provided by PEG 2000.

The influence of the incorporation of stearic acid-based or phosphatidylethanolamine-based modifiers into the membranes of PC/Chol liposomes on their pharmacokinetic behavior was assessed in rats. Five animals per group were used for these experiments. Since the disappearance of liposomes from the circulation is dose-dependent, the amount of lipid given was normalized to the weight of the animals. Each animal received 4.6 μmol of egg PC per kg of body weight. This amount corresponds to 9.2 μmol of total lipid per kg, which is consistent with the doses given in studies published by others. The amount of intact liposomes
FIGURE 3 Serum concentration-time profile subsequent to a 4.6 μmol/kg IV bolus injection of liposomes in 1 ml to rats. The amount of intact liposomes in the circulation was measured by fluorescence. The symbols indicate the averaged data, while the error bars indicate the standard deviation (n = 5). The lines represent the fit of the optimal models. Panel A: PC/Chol/PEG-PE liposomes (triangles), PC/Chol/PEG-SA liposomes (squares) and PC/Chol liposomes (diamonds). Panel B: PC/Chol/library-PE liposomes (triangles), PC/Chol/library-SA liposomes (squares) and PC/Chol liposomes (diamonds). The data for PC/Chol liposomes are the same for both panels.

In short, the peptide library can provide the liposomes with a better circulatory life than PEG under certain circumstances.

According to Torchilin et al. (1994), a long hydrophobic anchor is necessary if a relatively large polymer is to be used for surface grafting. In order for the polymer molecule to stay on the liposome surface, they argued, the energy involved in the interaction of the anchor with its phospholipid surroundings has to be higher than the energy of polymeric chain motion. The notable difference in pharmacokinetic behavior of liposomes modified with PEG-SA and PEG-PE is likely due to facile lipid exchange or dissociation of stearic acid relative to phosphatidylethanolamine. The library-PE modifier led to a much improved in vivo behavior of the liposomes, with a terminal half-life of 170 min (Fig. 3B). Unlike PEG-SA, library-SA improved the circulation half-life of PC/Chol liposomes when they were incorporated in the membrane as demonstrated by the terminal half-life of 55 min. This could well be a consequence of less favorable dissociation of the peptidyl modifier than PEG, which is in turn indicative of less hydration of the former than PEG.

In short, the peptide library can provide the liposomes with a better circulatory life than PEG under certain circumstances.

Our results with the peptide library are in sharp contrast to results obtained by others when grafting peptides on the surface of liposomes (Zalipsky et al., 1995). Long-circulating liposomes have recently been investigated as "platforms" for the presentation of biologically-relevant ligands such as peptides, which have a short biological half-life on their own. Liposomes containing 6% of PE-PEG 2000 were coupled to 5-residue peptides through the free extremities of the grafted PEG molecules. The incorporation of the peptides did, however, decrease the circulation half-life of these liposomes. As the number of grafted peptides per vesicle increased, the amount of liposomes present in blood after 24 h decreased, whereas the amount accumulated in the spleen increased. This clearly demonstrates an important difference in pharmacokinetic sequelae between the use of a hydrophilic peptide library for a prolong circulation and a peptide with a specific sequence for organ targeting when both are expressed on the liposome surface.

As an additional control, calcein (1 mg/kg) was administered to five rats. An intense orange color characteristic of calcein was excreted in urine 20-30 min after injection. Its disappearance was fast and almost mono-exponential. However, when rats received any of the liposome preparations, their urine did not have the characteristic calcein color. This corroborates the fact that the liposomes were eliminated through a different route than calcein and that very little calcein leaked out of the liposomes while they were in circulation. The latter was confirmed by the low fluorescence levels observed in serum in the absence of detergent throughout the experiments. It was observed that the terminal half-lives of liposomes coated with library-SA were approximately 5, 55 and 130 min for doses of 2.3, 4.6 and 9.2 μmol PC/kg.
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This work was supported by a research grant. 9213-ARG-8061, from the North Carolina Biotechnology Center. The study is presented also in memory of the late Professor Bruce W. Erickson. An abstract was presented at the annual meeting of the American Association of Pharmaceutical Scientists in San Francisco, USA, November 1998. A recent publication by Metselaar et al. (2003),

respectively (raw data not shown). This dose-dependant disappearance pattern is similar to that observed for conventional liposomes. The protection provided by the library delays liposome recognition, but does not appear to change the mechanism of elimination. Further investigation of this mechanism is warranted.

One common concern raised by the use of liposomes is their poor long-term stability. It has been observed that incorporation of fatty acids into liposomal layers, either as a result of phospholipid hydrolysis or exogenously added, increased the tendency of liposomes to aggregate (Kremer and Wiersema, 1977). Conventional liposomes as well as liposomes with added PEG-PE or library-PE showed only a minimal change in size and calcine entrapment after a year of storage at room temperature. However, liposomes with added PEG-SA or library-SA aggregated after just a few weeks. The fact that all the formulations comprising a stearic acid-based membrane modifier aggregated much earlier than the other formulations indicates that stearic acid may not strongly incorporate into the membranes, and may provoke membrane defects when it dissociates from it.

CONCLUSIONS

In conclusion, a new family of liposome surface modifiers was prepared and characterized. In vivo experiments showed that some of the amphiphilic compounds prepared for this study exerted an important effect on the pharmacokinetics of liposomes. The pharmacokinetic profile of these modified liposomes is greater flexibility of use than PEG-PE, allowing to tailor the pharmacokinetic profile of the liposomes depending on their intended application.

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References


