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THE EFFECTS OF CHARGE AND SIZE ON THE INTERACTION OF UNILAMELLAR LIPOSOMES WITH MACROPHAGES

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The effect of the positive surface charge of unilamellar liposomes on the kinetics of their interaction with rat peritoneal macrophages was investigated using three sizes of liposomes: small unilamellar vesicles (approx. 25 nm diameter), prepared by sonication, and large unilamellar vesicles (100 nm and 160 nm diameter), prepared by the Lipoprep dialysis method. Charge was varied by changing the proportion of stearylamine added to the liposomal lipids (egg phosphatidylcholine and cholesterol, molar ratio 10:2.5). Increasing the stearylamine content of large unilamellar vesicles over a range of 0–25 mol% enhanced the initial rate of vesicle-cell interaction from 0.1 to 1.4 μg lipid/min per 10^6 cells, and the maximal association from 5 to 110 μg lipid/10^6 cells. Cell viability was greater than 90% for cells incubated with large liposomes containing up to 15 mol% stearylamine but decreased to less than 50% at stearylamine proportions greater than 20 mol%. Similar results were obtained with small unilamellar vesicles except that the initial rate of interaction and the maximal association were less sensitive to stearylamine content. The initial rate of interaction, with increasing stearylamine up to 25 mol%, ranged from 0.5 to 0.7 μg lipid/min per 10^6 cells, and the maximal association ranged from 20 to 70 μg lipid/10^6 cells. A comparison of the number and entrapped aqueous volume of small and large vesicles containing 15 mol% stearylamine revealed that although the number of large vesicles associated was 100-fold less than the number of small vesicles, the total entrapped aqueous volume introduced into the cells by large vesicles was 10-fold greater. When cytochalasin B, a known inhibitor of phagocytosis, was present in the medium, the cellular association of C_8-LUV was reduced approx. 25% but association of SUV increased approx. 10–30%. Modification of small unilamellar vesicles with an amino mannosyl derivative of cholesterol did not increase their cellular interaction over that of the corresponding stearylamine liposomes, indicating that cell binding induced by this glycolipid may be due to the positive charge of the amine group on the sugar moiety. The results demonstrate that the degree of liposome-cell interaction with macrophages can be improved by increasing the degree of positive surface charge using stearylamine. Additionally, the delivery of aqueous drugs to cells can be further improved using large unilamellar vesicles because of their greater internal volume. This sensitivity of macrophages to vesicle charge and size can be used either to increase or reduce liposome uptake significantly by this cell type.

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Abbreviations: SUV, small unilamellar vesicles; C_7-LUV, large unilamellar vesicles (approx. 100 nm diameter) prepared by dialysis using the heptyl-β-D-glucopyranoside detergent; C_8-LUV, large unilamellar vesicles (approx. 160 nm diameter) prepared by dialysis using the octyl-β-D-glucopyranoside detergent; DTPA, diethylenetriaminepentaacetic acid; PC, phosphatidylcholine; DSPC, L-α-distearoylphosphatidylcholine; NManChol, 6-(cholest-5-en-3-β-xyloxy)hexyl-6-amino-6-deoxy-1-thio-α-D-mannopyranoside.

Introduction

Liposomes are potentially useful as a carrier system to deliver pharmacologically active agents to cells, especially if they can be directed to specific cell types in vivo. We have studied various types of liposomes in order to identify the factors important to cell targeting. Rahman et al. [1], using liposomes differing in size and lipid composition, studied the uptake characteristics of the liver parenchymal and Kupffer cells in vivo. It was found that large multilamellar liposomes were mainly taken up by the Kupffer cells while, unexpectedly, small unilamellar liposomes were less effectively taken up. Because of the known phagocytic characteristics of reticuloendothelial cells (e.g., Kupffer cells), it is essential to understand better the factors regulating the association or interaction of liposomes with these cells leading to subsequent uptake of the liposomes. The present study systematically examines the roles of liposome size and positive surface charge in the interaction of liposomes and rat peritoneal macrophages, using small unilamellar vesicles (SUV) of approx. 25 nm in diameter and large unilamellar vesicles (LUV) of approx. 100 and 160 nm in diameter. The degree of surface charge was varied by changing the proportion of stearylamine in the lipid mixture. Diethylenetriaminepentaacetic acid (DTPA), radiolabeled with ^{59}Fe (^{59}Fe-DTPA) was used as an aqueous marker for liposomes because nonencapsulated ^{59}Fe-DTPA is not taken up by cells, and because of the possible therapeutic relevance of DTPA as a metal chelating drug.

Materials and Methods

Materials

Lyophilized egg phosphatidylcholine (PC) and synthetic L-α-distearoylphosphatidylcholine were from Avanti Polar Lipids, Birmingham, AL. Cholesterol (99.1% pure) and cytochalasin B were from Sigma, St. Louis, MO. Stearylamine (twice recrystallized) was from K & K Labs, Plainview, NJ. *n*-Octyl- and *n*-heptyl-β-D-glucopyranoside were from Calbiochem, La Jolla, CA. The amino mannose-modified cholesterol, 6-(cholest-5-en-3-β-xyloxy)hexyl-6-amino-6-deoxy-1-thio-α-D-mannopyranoside (NManChol) was a gift from Dr. T.Y. Shen, Merck, Sharpe and Dohme, Rahway, NJ. DTPA (Calcium Chel 330) was from the U.S. Department of Energy, Washington, DC. $^{59}FeCl_3$ was from New England Nuclear, Boston, MA. [^{3}H]Cholesterol and [^{14}C]phosphatidylcholine were from Amersham, Arlington Heights, IL. Tissue culture medium, antibiotics, and fetal bovine serum were obtained from Flow Laboratories, Inc., McLean, VA. Sephadex G-50 medium and Sephacryl S-1000 were from Pharmacia, Piscataway, NJ. All other reagents used were of analytical grade.

Methods

Preparation of liposomes. Unilamellar liposomes were usually prepared with the following lipid composition: egg PC/cholesterol/stearylamine in a molar ratio of 10 : 2.5 : X (where $X = 0$–2.5). Percent stearylamine refers to the mole% relative to phosphatidylcholine. In the preparation of neutral liposomes, stearylamine was either omitted from the lipid mixture or replaced by 15 mol% stearic acid methyl ester. Specific liposome preparations carrying a negative surface charge were prepared with 15 mol% phosphatidylserine or phosphatidic acid in place of stearylamine. The lipids were dissolved in 1 : 1 (v/v) chloroform/methanol and, in the case of 'detergent dialysis' liposomes (see below), an appropriate amount of detergent was added. The solvent was then removed by rotary evaporation at 40°C for 60 min, and the lipids were further dried under a stream of nitrogen. The lipid film was hydrated in 4 ml of 0.15 M NaCl, 10 mM Tris-HCl (pH 7.4) buffer containing the ^{59}Fe-DTPA complex as an aqueous label. ^{59}Fe-DTPA was prepared by the complexation of 2 μM $FeCl_3 \cdot 6H_2O$ and 4 μM DTPA with the addition of 0.2 mCi radiolabeled ferric chloride.

(A) Large liposomes prepared by 'detergent dialysis': large unilamellar vesicles were prepared by the Lipoprep dialysis method as described in Refs. 2 and 3 using C_8-glucoside detergent to prepare liposomes of 160 nm mean diameter (C_8-LUV) and C_7-glucoside detergent to prepare liposomes of mean diameter 100 nm (C_7-LUV). The molar ratio of total lipid to detergent was 0.2 for C_8- and 0.13 for C_7-glucoside. The dialysis was performed as described by Schwendener et al. [3] for 60 min at 40°C in a closed outer dialysate circuit of 40 ml

of the Tris-HCl buffer containing unlabeled Fe-DTPA to minimize the loss of the aqueous marker. Lipid loss due to dialysis was approx. 5–10%. Nonencapsulated ^{59}Fe-DTPA and residual detergent were removed by gel chromatography on a Sephadex G-50 medium column (40 × 1.5 cm, 1 ml/min flow rate) using 0.15 M NaCl/10 mM Tris-HCl (pH 7.4) as the eluant. Fractions containing the liposomes were pooled in 10.0 ml to yield a solution with a PC concentration of 2 mg per ml. The amount of residual detergent in the liposome preparations was 1 ± 0.15%.

(B) Small liposomes prepared by sonication: small unilamellar vesicles were prepared by ultrasonication of lipid dispersions, with a Biosonic III microtip sonicator for 60 min at 4°C. Multilamellar vesicles and metal impurities from the sonicator tip were removed by ultracentrifugation at 100000 × g for 60 min. The supernatant SUV were chromatographed on a Sephadex G-50 medium column to remove nonencapsulated ^{59}Fe-DTPA, as described for the detergent dialysis liposomes.

(C) Small liposomes modified with amino mannose: small unilamellar vesicles modified with an amino mannosyl derivative of cholesterol and containing either L-α-distearoyl-PC (DSPC) or egg PC were prepared with the composition PC/cholesterol/NManChol in the mole ratio 10 : 2.5 : 2.5 or PC/cholesterol/NManChol/stearylamine in the mole ratio 10 : 2.5 : 1.5 : 1.5. Liposomes containing DSPC were prepared as described for sonicated liposomes with the following modifications. The lipids in organic solvents were dried at 65°C for 60 min. To facilitate the dispersion of the lipids in the labeled Tris-HCl buffer, the suspensions were briefly sonicated in a bath-type sonicator. The lipid mixture was then sonicated using the probe-type sonicator for 30 min at 65°C and then centrifuged at 50000 × g for 60 min. When egg PC was used in place of DSPC, the liposomes were sonicated at 4°C. In a few experiments [^{3}H]inulin was used as an aqueous liposomal marker. All preparations were used immediately after the removal of the nonencapsulated radiomarker by column chromatography.

Determination of ^{59}Fe-DTPA leakage. Freshly prepared SUV and C_8-LUV (1 mg PC in 0.5 ml) were mixed with 2.0 ml William's E medium and 0.5 ml of 0.15 M NaCl, 10 mM Tris-HCl (pH 7.4) (identical ratio as used in the cell incubation experiments) and put into small dialysis bags. Each dialysis bag was placed into an individual conical centrifuge tube containing 27 ml of William's E medium and 13 ml of the Tris-HCl buffer and incubated with gentle rocking at 37°C for various time intervals. Following incubation, the bags were removed and rinsed with buffer; the bag plus its contents was placed in a counting vial; and the radioactivity was determined along with that of the dialysate. Leakage from the liposomes was expressed as the percent distribution of radioactivity between the bag and the dialysate. The release of free ^{59}Fe-DTPA from the dialysis bags was used as a control.

Liposome size determination. Liposome size and homogeneity were determined with [^{3}H]cholesterol or [^{14}C]PC-labeled SUV, C_7-, and C_8-LUV preparations by column chromatography on an 80 × 0.9 cm Sephacryl S-1000 column, at a flow rate of 3.5 ml per h and a 0.5 ml fraction volume, according to the method of Nozaki et al. [4]. Detergent dialysis liposomes of known sizes (180 and 80 nm) [3], prepared with egg PC as the sole lipid, were chromatographed on the same column as standards.

Preparation of macrophages. Peritoneal macrophages were obtained by peritoneal lavage of unstimulated rats; $4.5 \cdot 10^6$ cells, suspended in William's medium E supplemented with 10% fetal calf serum, 50 I.U./ml penicillin and 50 μg/ml streptomycin, were plated in 60-mm tissue culture dishes. The adhered monolayer of viable macrophages was then maintained overnight in a humidified CO_2 incubator before use. Prior to incubation with the liposomes, the cells were washed twice with 2.0 ml phospate-buffered saline; then 2.0 ml serum-free William's medium E plus 0.5 ml of the Tris-HCl buffer was added. Cell viability was determined by Trypan blue exclusion. Cells were removed from the control plates by treatment with 2.5 mM EDTA for 30 min at 4°C and were counted using a haemocytometer. An adhered monolayer of $1.8 \pm 0.2 \cdot 10^6$ cells per plate was used in each incubation. To study the effect of cytochalasin B on the cell-association of SUV and LUV, the inhibitor in dimethyl sulfoxide was added to the incubation medium at a final

concentration of $3 \cdot 10^{-5}$ M cytochalasin B and 0.3% dimethyl sulfoxide, and the cells preincubated for 30 min at 37°C before the addition of the liposomes.

Association of liposomes with macrophages. Freshly chromatographed liposomes (1 mg PC in 0.5 ml) labeled with ^{59}Fe-DTPA were added to each macrophage culture of $1.8 \pm 0.2 \cdot 10^6$ cells. The plates were incubated with gentle rocking at 37°C in a CO_2 incubator. At selected time intervals from 20 to 240 min, the incubation medium was removed and the cells were washed with phosphate-buffered saline. Nonadhered cells in the combined medium and washes were removed by centrifugation and the radioactivity in the adhered and nonadhered cell fractions was determined separately. The adhered cells were treated with trypsin and removed by scraping before determination of their radioactivity. Liposome-cell association was expressed as the percentage of cell-associated radioactivity relative to the total radioactivity recovered.

Results

Liposome characterization

Preparations of SUV, C_7-, and C_8-LUV were analyzed by Sephacryl S-1000 gel chromatography to determine their size and homogeneity. Fig. 1 shows the elution patterns of liposome preparations containing 15 mol% stearylamine, as used in the macrophage incubation experiments. The narrow elution peak of each liposome preparation indicates a homogeneous population. Extrapolation from the elution characteristics of identically prepared egg PC liposomes of well characterized size as described by Schwendener et al. [3], identifies the vesicle sizes of the three populations as 160 nm for the C_8-LUV, 100 nm for the C_7-LUV, and 25 nm for the SUV.

Because the cell-association of the liposomes is determined by analysis of an entrapped aqueous radiomarker, and because the liposomes are potentially useful as a delivery system for aqueous drugs, the entrapment efficiencies and leakage of ^{59}Fe-DTPA were determined for the three liposome size classes. The entrapment efficiencies were calculated to be $17.7 \pm 2.5\%$ for C_8-LUV, $7.8 \pm 1.5\%$ for C_7-LUV, and $1.1 \pm 0.4\%$ for SUV. Leakage of

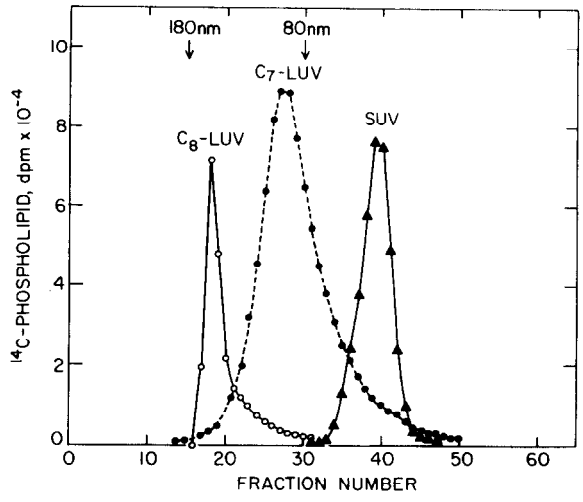

Fig. 1. Sephacryl S-1000 elution patterns for C_8-LUV, C_7-LUV and SUV. The lipid composition of all liposomes was egg PC/cholesterol/SA of 10/2.5/1.5 mol%. Each preparation was chromatographed separately on a 80×0.9 cm column with a flow rate of 3.5 ml/h and void volume of 10.5 ml. The elution peaks of egg PC detergent dialysis liposomes of known sizes (180 nm and 80 nm mean diameter) are indicated by arrows. SA, stearylamine.

the entrapped radiomarker was more pronounced for the small liposomes compared with the LUV; C_8-LUV retained more than 95% of the entrapped marker after 120-min incubation compared with 90% for the SUV. This difference could perhaps be due to the larger total lipid bilayer surface relative to volume of SUV which would increase the diffusion rate of the ^{59}Fe-DTPA.

Liposome-macrophage interactions

The effect of increasing the net positive surface charge of SUV and LUV on their interaction with rat peritoneal macrophages is shown in Fig. 2. Increasing the degree of positive charge in SUV, C_7-, and C_8-LUV increased their cell association. SUV containing 15 mol% of stearic acid methyl ester (methyl stearate), an uncharged molecule retaining the long hydrocarbon chain of stearylamine, behaved similarly to the neutral (0 mol% stearylamine) SUV, indicating that the positively charged amine group is alone responsible for the increased liposome-cell interactions shown in Fig. 2. Under our experimental conditions, the degree of cell-association of liposomes bearing negative surface charges introduced by 15 mol% phos-

phatidic acid or phosphatidylserine was similar to that of their neutral counterparts. Control incubations of cells with free ^{59}Fe-DTPA or [^{3}H]inulin showed negligible cellular interaction of these two markers (less than 0.1% and 0.4%, respectively). Cell viability as assessed by Trypan blue exclusion was excellent and remained greater than 90% after 240 min incubations with either SUV or LUV containing up to 15 mol% stearylamine. With liposomes containing stearylamine proportions greater than 20 mol%, cell viability was reduced to less than 50% after 60 min.

The effect of increased charge on liposome-cell association expressed in terms of cell associated lipid was similar for both small and large unilamellar vesicles; but SUV association appeared to be less sensitive to stearylamine content than LUV association (Fig. 2). C_8-LUV showed a substantial increase in cell association with increasing stearylamine content and virtually no cell interaction when stearylamine was not present. In con-

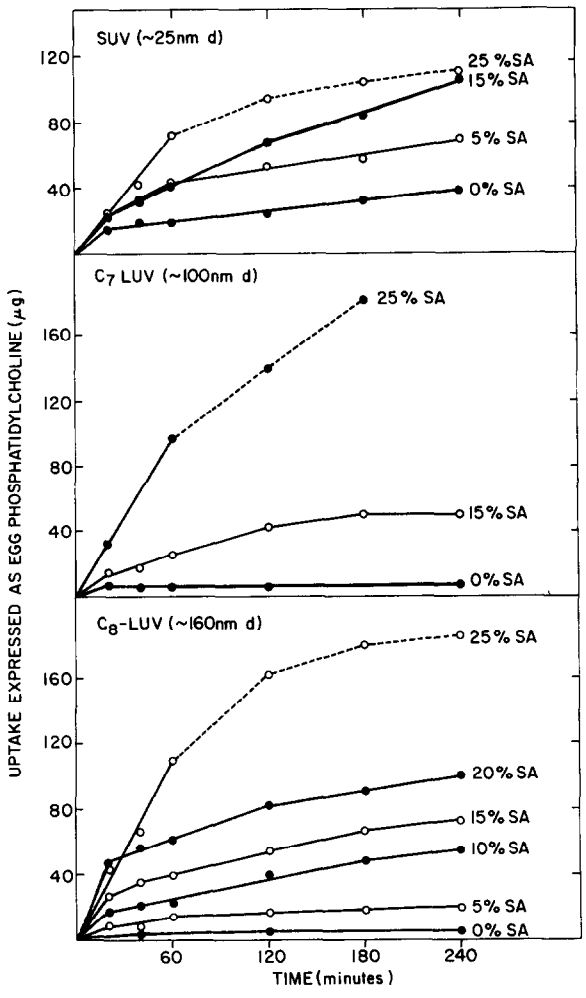

Fig. 2. Effect of increasing positive surface charge on the lipid-cell association of SUV (upper panel), C_7-LUV (middle panel), and C_8-LUV (lower panel) by rat peritoneal macrophages. Positive charge was increased by changing the proportion of stearylamine (SA) added to the liposomal lipids (egg PC and cholesterol, molar ratio 10:2.5). Percent stearylamine is the mol% relative to phosphatidylcholine. Vesicles were labeled in the aqueous phase with ^{59}Fe-DTPA; $1.8 \pm 0.2 \cdot 10^6$ macrophages were incubated with liposomes containing 1 mg total lipid at 37°C for 20–240 min. Each point represents the mean value of duplicate incubations in 2–3 individual experiments. Note that with liposomes containing stearylamine proportions greater than 20 mol%, cell viability was reduced to less than 50% after 60 min. To avoid any ambiguity due to this reduced cell viability, portions of the curve after 60 min are represented by dashed lines.

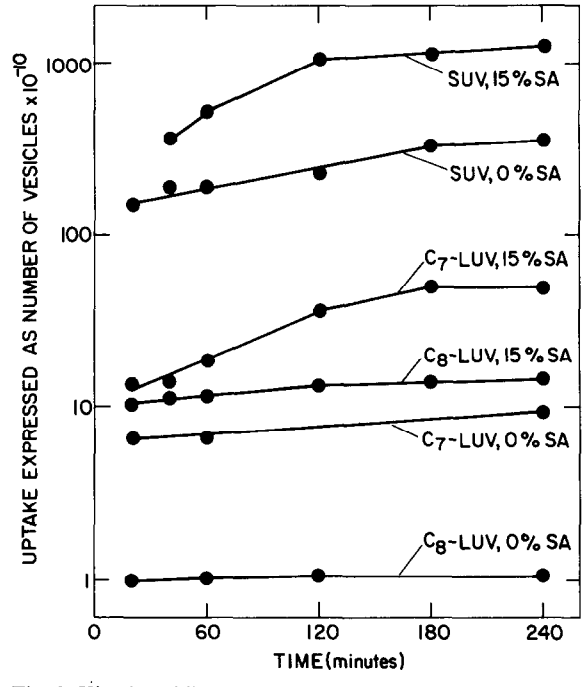

Fig. 3. Kinetics of liposome-cell association expressed as number of liposomes associated with rat peritoneal macrophages. Neutral (0 mol% stearylamine (SA)) and positively charged (15 mol% SA) SUV, C_7-LUV, and C_8-LUV are compared. Percentages are relative to phosphatidylcholine. The numbers of vesicles were calculated for spherial vesicles based on the following assumptions: (a) average diameters of 160 nm for C_8-LUV, 100 nm for C_7-LUV, and 25 nm for SUV; and (b) an average M_r of 750 for egg PC. Incubation conditions are as described in Fig. 2.

trast, a significant portion of neutral SUV were found cell-associated, but the overall magnitude of liposome-cell association with increasing stearylamine content was not as pronounced. Initial rates of liposome-cell association as determined in the first 20 min of incubation also varied depending on the liposome size. For the C_8-LUV, the initial rates increased from 0.1 to 1.4 μg lipid/min per 10^6 cells for increases in stearylamine content from 0 to 25 mol% whereas SUV rates increased only from 0.5 to 0.7 μg lipid/min per 10^6 cells over the same range of stearylamine content.

The effect of liposome size on cellular interaction becomes more evident when the number of vesicles and the volume of their cell-associated contents are calculated. Fig. 3 compares the total numbers of vesicles that become cell associated for SUV, C_7-LUV, and C_8-LUV containing 15 mol% stearylamine. An approximately 100-fold greater number of positively charged SUV become cell-associated than C_8-LUV's. Also for the neutral liposomes, the number of cell-associated SUV is approx. 100-times greater than the number of C_8-LUV, indicating that the 100-times greater concentration of small liposomes per unit weight of lipid in the system may compensate for the lack of charge. Fig. 4 compares the liposome-cell association based on the entrapped aqueous volume introduced to the cells and illustrates an effective advantage of large size liposomes. Neutral C_8-LUV introduce an identical aqueous volume as positively charged SUV into macrophages and this volume is increased by a factor of 10 when charged C_8-LUV are utilized. This result renders the large liposomes an especially useful means of introducing trapped aqueous compounds into cells, assuming that the liposomes are taken up intact by the cells.

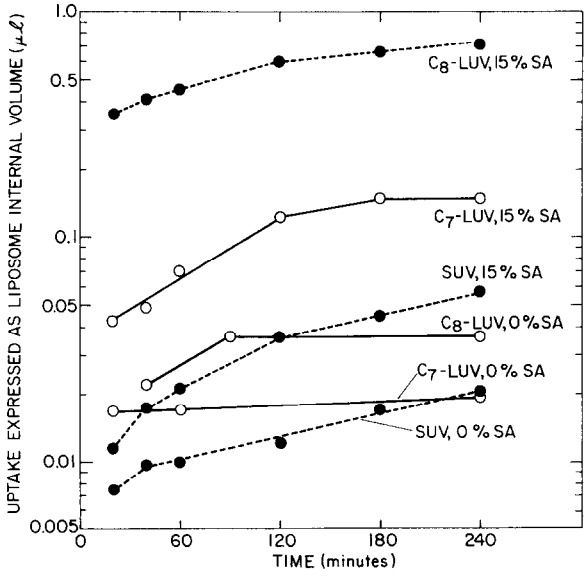

Fig. 4. Liposome-cell association expressed as internal aqueous volume (μl) per $1.8 \pm 0.2 \cdot 10^6$ macrophages. Neutral (0 mol% stearylamine (SA)) and positively charged (15 mol% SA) SUV, C_7-LUV, and C_8-LUV are compared. Percentages are relative to phosphatidylcholine. Calculations are based on assumptions given in Fig. 3 legend and a bilayer thickness of 4 nm. Incubation conditions are as described in Fig. 2.

Effects of temperature and cytochalasin B on liposome uptake

The effects of temperature and of cytochalasin B, a known inhibitor of phagocytosis, on the cell association of SUV and LUV by macrophages was studied. Incubations at 4°C compared with 37°C resulted in approximately a 70% reduction in the cell-association of both SUV and LUV. Liposome size appeared to be a factor affecting the action of cytochalasin B. Incubation of the macrophages at

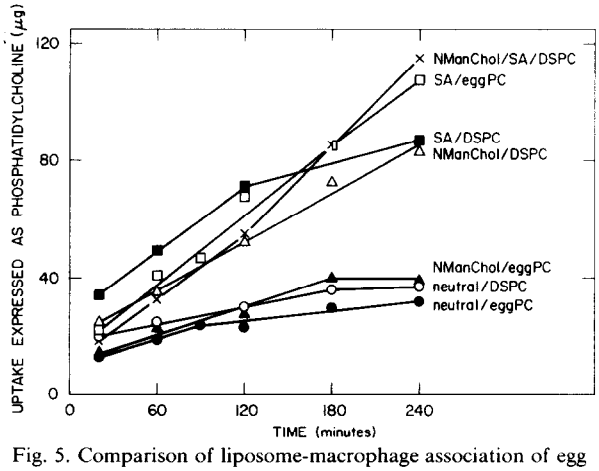

Fig. 5. Comparison of liposome-macrophage association of egg PC and L-α-distearoylphosphatidylcholine SUV modified with NManChol and stearylamine (SA). All liposome preparations contained either egg PC or DSPC and cholesterol in the molar ratio 10:2.5. The SUV were modified with either 25 mol% NManChol, 15 mol% stearylamine, or a combination of 15 mol% of both. Percentages are relative to phosphatidylcholine. Incubation conditions are as described in Fig. 2 legend.

37°C in the presence of cytochalasin B reduced the cell association of C_8-LUV approx. 25% but increased the association of SUV approx. 10–30%.

Amino mannose modified liposomes

In an additional series of experiments, SUV were modified by the addition of an amino mannosyl derivative of cholesterol instead of stearylamine to compare the efficacy of possible receptor-mediated interaction elicited by the glycolipid [5] with the charge-mediated interaction elicited by stearylamine. The results, summarized in Fig. 5, show that cellular interaction of liposomes containing NManChol is no greater than that of the corresponding stearylamine liposomes. When egg PC is replaced by DSPC, the change in the membrane fluidity appears to alter the characteristics of cellular interaction of the liposomes containing the glycolipid. An analogous effect was not observed with stearylamine-modified liposomes. In addition, liposomes composed of equal parts of stearylamine and NManChol do not show any significant enhancement in vesicle-cell interactions.

Discussion

The complex interactions of liposomes with phagocytic cells can in general be described by the following simplified steps: (a) stable adsorption to the cell surface, (b) cellular uptake of intact vesicles by an energy dependent mechanism, and (c) lysosomal degradation of the liposomes and their contents. Fusion, lipid exchange, and a combination of these mechanisms also can occur [6,7]. Liposome adsorption to the cell surface seems to be the rate-limiting step, since it can be assumed that stably adsorbed vesicles are more susceptible to subsequent uptake than vesicles that are only loosely interacting with the cell surface. The present studies show that the incorporation of positive surface charges using stearylamine into liposomes enhances this initial interaction. This finding has also been reported by Magee et al. [8] with HeLa cells, which are less phagocytic than macrophages. In a similar investigation with rabbit thymocytes, Roozemond and Urli [9] showed a strong increase in liposome-cell adsorption with positively charged vesicles compared with neutral or negatively charged vesicles. In a study in which SUV with different charges were incubated with murine L1210 cells, Jansons et al. [10] found a 20-fold higher cell-association of positively charged liposomes than of neutral or negatively charged vesicles. The binding capacity of liposomes containing stearylamine to erythrocytes was investigated by Martin and McDonald [11] who found that stearylamine is essential for cell binding but that hemolysis can occur at stearylamine amounts of 0.5 mol parts in an electrolyte-free system.

Our results show a strong, interdependent influence of vesicle charge and size on lipsome-macrophage interactions. They demonstrate that the efficiency of liposome-cell association can be improved by increasing the degree of positive surface charge using stearylamine up to a maximum of 15 mol% while retaining a high cell viability. Above 20 mol% stearylamine, cell viability was significantly reduced. This side effect of stearylamine is probably less pronounced under physiological conditions, but should be considered in the selection of a suitable stearylamine content for optimal cell interaction. Because liposomes made with stearic acid methyl ester, an uncharged molecule retaining the long hydrocarbon chain of stearylamine, behaved similarly to neutral liposomes, we can rule out characteristics other than charge as affecting the interactions of stearylamine liposomes with cells. The absolute charge of the liposomes containing stearylamine was not determined. Hauser and Guyer [12] have demonstrated that long-chain fatty acids incorporated into liposomes are not fully ionized at neutral pH although they observed a linear increase in electrophoretic mobility (net liposome charge) with increasing amounts of the acidic component. It is logical to assume that stearylamine would behave in a similar manner, suggesting that although the stearylamine may not be fully ionized, a linear increase in net liposome charge with increasing amounts of stearylamine would be obtained.

The effect of increased charge on liposome-cell association was similar for both small and large unilamellar vesicles, but SUV association was less sensitive to stearylamine content than was the association of LUV. This phenomenon may result from the approx. 100-fold greater concentration of small liposomes per unit weight of lipid, or vesicle

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The effect of increased charge on liposome-cell association was similar for both small and large unilamellar vesicles, but SUV association was less sensitive to stearylamine content than was the association of LUV. This phenomenon may result from the approx. 100-fold greater concentration of small liposomes per unit weight of lipid, or vesicle

greater internal volume. Targeting of liposomes to cells other than those of the reticuloendothelial system is a difficult problem. The present study shows that the sensitivity of macrophages to vesicles of different charge and size can be used either to significantly reduce or increase liposome interaction with cells of the reticuloendothelial system. Comparative studies are currently under way to assess the effect of serum on liposome-cell interactions as well as the relative importance of various liposomal characteristics (e.g., size, charge, and site-specific groups) in uptake by nonreitculoendothelial cells (e.g. hepatocytes).

Acknowledgments

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