Biochimica et Biophysica Acta 814 (1985) 256–264
Elsevier

BBA 72516

Modification of vesicle surfaces with amphiphilic sterols. Effect on permeability and in vivo tissue distribution

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(Received November 19th, 1984)

Key words: Membrane permeability; Liposome stability; Cholesterol derivative; Tissue distribution

In this paper, we describe the permeability of vesicles prepared with various synthetic cholesterol derivatives. Cholesterol derivatives with side-chains ending in hydroxyl groups reduced the permeability of unilamellar vesicles. However, addition of cholesterol derivatives with terminal amino groups makes the vesicles more permeable. Vesicles prepared with a short-chain amino-cholesterol derivative were found to be less permeable in phosphate-buffered saline, but not in bovine serum, while long-chain amino-cholesterol-containing vesicles were very permeable in both media. Studies in vivo indicate a rapid clearance rate for intravenously administered amino-cholesterol-containing vesicles with a concomitant increase in liver uptake. However, no difference was found in either the clearance or tissue distribution of control vesicles and the less permeable hydroxyl-cholesterol-containing vesicles.

Introduction

Phospholipid bilayer vesicles, originally developed by Bangham et al. as model membranes [1], have become a focus of research interest in recent years because of their potential as carriers for a variety of biologically active materials. Thus, for example, enzymes [2], chelating agents [3], viral nucleic acids [4] anti-tumor drugs [5], antibiotics [6], immunogens [7] and hormones [8] have been successfully encapsulated within liposome systems. A major obstacle, however, to the use of circulating liposome carriers has been their rapid uptake and removal by the liver.

Several approaches have been taken to deliver selectively liposome-encapsulated materials to specific organs or types of tissues. For example, it has been shown that vesicle size, altered by sonication, can result in an increased uptake by kidney [9]. Modification of lipid acyl groups, to alter the liposome phase-transition temperature, can induce the preferential release of encapsulated materials at sites of mild hyperthermia [10]. Liposomes, sensitive to pH, have been prepared and shown to release materials at sites of inflammation and infection, where the pH is slightly below the physiological level [11].

Alternatively, one can change the surface properties of liposomes so that they well interact specifically with cell surface groups on the targeted tissue. Successful demonstrations of this approach include the attachment of tumor cell-specific antibodies to a vesicle surface [12] and incorporation of lipid-containing charged groups [13–15] or carbohydrates [5,15,16], which alter the liposome surface and tissue distribution.

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Abbreviations: PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine.

In previous work from this laboratory, it was shown that carbohydrate-containing cholesterol analogues incorporated into liposomes can effect both stability and tissue distribution [17–19]. In order to obtain a better understanding of the basis for these effects, we have prepared a series of cholesterol derivatives, varying in their length, charge and polarity. In this report, we compare the permeability and tissue distribution of phospholipid vesicles containing these derivatives.

Materials and Methods

Reagents. 1,2-Dipalmitoyl- and 1,2-distearoyl-*sn*-glycero-3-phosphocholine, stearylamine, cholesterol and cholesteryl-*p*-toluenesulfonate were obtained from Sigma Chemical Co., St. Louis, MO. Desferroxamine was purchased from CIBA Pharmaceutical Co., Summit, NJ. Amersham/Searle Corp., Arlington Heights, IL was the source of radioactive iron in the form of $^{59}FeCl_3$ in 0.1 M HCl (1 mCi/ml) and D-[1-^{3}H]glucose (4.5 Ci/mmol). Sephadex G-50 fine (20–80 μm diameter) was obtained from Pharmacia, Uppsala, Sweden.

Synthesis. The following chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, and purified by distillation just before use: ethylene glycol (b.p. 65–68°C/0.5 mmHg); triethylene glycol (b.p. 113–115°C/0.01 mmHg); ethylenediamine (b.p. 28–30°C/1 mmHg); 1,6-hexanediamine (sublimated, m.p. 38–40°C); *N*-(2-aminoethyl)-1,3-propanediamine (used as received); and triethylenetetramine (b.p. 136–137°C/0.5 mmHg). Dioxane was purified by refluxing over lithium aluminum hydride for several hours followed by distillation prior to use. Thin-layer chromatography (TLC) was performed on Merck (Darmstadt, F.R.G.) precoated silica gel 60/Kieselguhr F-254 plates and visualized with I_2 or H_2SO_4/charring of ninhydrin spray. Infrared spectra were recorded with a Beckman IR-4240 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Varian XL-200 MHz spectrometer. Optical rotations were measured with a JASCO DIP-181 digital polarimeter.

The method for the preparation of cholesterol derivatives in this paper is essentially the same as that for the synthesis of cholesterol derivative II (3,6,9-trioxa-octan-1-ol-cholesteryl-3ϵ-ol) as reported recently by us [20]. The isolated products were characterized by infrared spectroscopy, NMR and TLC and were pure enough to be used directly without further purification. The reactions of cholesteryl-*p*-toluenesulfonate with substrates is summarized in Table I.

Preparation of vesicles. Liposomes containing desferroxamine with a trace amount of ^{59}Fe in the aqueous compartment were prepared according to methods described by Patel et al. [20]. DPPC or DSPC, cholesterol and stearylamine, in the molar ratio of 1.54 : 1.0 : 0.43, respectively, were dried in a round-bottom flask. When liposomes were prepared with various synthetic derivatives, half of their cholesterol amount was replaced with individual cholesterol derivative. An aqueous phase containing 75 mg desferroxamine and a trace amount of ^{59}Fe/ml water was added in the flask and stirred for 10 min. The weight ratio of lipids to desferroxamine was approx. 1 : 2. Liposomes containing DPPC were prepared at 50°C and DSPC at 60°C. Small unilamellar vesicles were prepared by sonication of the multilamellar vesicles for 30 min in a water-bath sonicator (Model G11 2SPIT, Laboratory Supplies Co., Hickville, NY). Nonencapsulated ^{59}Fe-tagged desferroxamine was removed from the vesicles by the method of Fry et al. [21]. Liposomes containing [^{3}H]glucose were prepared by a method similar to that for liposome-encapsulated desferroxamine.

The permeability of the vesicles was assessed by the dialysis method. Vesicles, approx. 8–15 mg total lipids, were suspended in 1 ml of either phosphate-buffered saline or fetal calf serum and placed in a dialysis bag. The dialysis bag was placed in 40 ml phosphate-buffered saline. Dialysis was carried out at 37°C; aliquots of the dialysis solution were removed at various times and the percent leakage of the ^{59}Fe-tagged desferroxamine was determined using a Beckman Biogamma counter.

Injection of vesicles and analysis of radioactivity in mouse tissue samples. Vesicles, approx. 1.5–2 mg total lipids, were injected by a single intravenous injection of 0.2 ml into 2–3-month old Swiss Webster female mice. Groups of mice (four mice per group) were killed 2 h after injection. Blood from

TABLE I

SUMMARY OF REACTIONS OF CHOLESTERYL-*p*-TOLUENESULFONATE WITH SUBSTRATES

Reactions were carried out with 4 mmol cholesteryl-*p*-toluenesulfonate and 25–30 molar equivalents of substrate in refluxing dioxane for 2–4 h under N_2 with stirring [20]. All isolated produces were colorless syrup, except I, m.p. 103–104°C (petroleum ether, 30–60°C) Lit. [40] 93–105°C. The infrared spectrum of I was taken in nujol mull. Isolated products showed a single homogeneous spot on TLC and complete disappearance of starting material cholesteryl-*p*-toluenesulfonate and the substrate. TLC was performed in solvent system for products: I (R_F 0.30) in chloroform, II (R_F 0.43) in chloroform/ethylacetate (1:1, v/v), III (R_F 0.43) in chloroform/methanol/ammonium hydroxide (2:2:1, v/v), IV (R_F 0.77) in chloroform/methanol/ammonium hydroxide (3:2:1, v/v), V (R_F 0.58) and VI (R_F 0.79) in chloroform/methanol/ammonium hydroxide (2:2:1, v/v). For product VI, no attempt was made to obtain maximum yield. Abbreviations used are s = singlet, d = doublet, m = multiplet, and b = broad. Products I–VI have similar chemical shifts at 4.50–4.70 (m, 1H; C-6), 9.00–9.07 (s, 3H; C-19), 9.07–9.15 (d, 3H; C-21), 9.12–9.21 (d, 6H; C-26 and C-27), and 9.28–8.38 (s, 3H; C-18).

Products (% isolated yield)	Specific rotation $[\alpha]_D^{25}$ ($CHCl_3$)	Infrared spectrum (neat) ν_{max} (cm^{-1})	^{1}H-NMR ($CDCl_3$, τ)
I (81)	−30.95°	3470 (medium-weak) 800 (weak) 1110, 1060 (medium-weak)	6.78 (m,b,1H,OH) 6.28,6.39 (m,4H,CH_2O)
II (92)	−25.38°	3460 (strong) 1640 (weak) 865 (medium-strong) 1112 (strong)	6.84 (m,1H,OH) 6.35,6.38 (s,12H.CH_2O)
III (75)	−6.56°	3310, 3370 (medium-weak) 1615 (weak) 800 (medium) 1115 (medium-weak)	7.34,7.37 (m,4H,CH_2N)
IV (78)	−7.25°	3380, 3310 (weak) 1665 (medium,sharp) 875 (medium,sharp) 1125 (medium-sharp)	4.74 (b,1H,NH) 6.29 (s,12H,CH_2N)
V (65)	−5.0°	3300 (medium-weak) 1665 (medium-sharp) 800 (medium-weak) 1123 (medium-weak)	4.74 (m,b,1H,NH) 6.37,6.38 (s,1OH,CH_2N)
VI (25)	−13.85°	3300 (medium-weak) 1580 (weak) 800 (medium) 1120 (medium-weak)	6.35,6.38 (s,12H,CH_2N)

the jugular vein was collected. The amount of vesicle uptake in the total blood was calculated by presuming that blood comprises 7.3% of the total weight of the animals [17]. For bone marrow, two rear tibias from each mouse were counted for radioactivity. The amount of vesicle uptake in the total bone marrow was estimated by multiplying the radioactivity in the two tibial segments by a factor of 44 [22]. Other tissues were removed and radioactivity was determined with a gamma counter.

Results

The chemical structures of the various cholesterol derivatives are shown in Fig. 1. Derivatives I and II have a terminal hydroxyl group and III, IV, V and VI have terminal primary amine groups and different numbers of secondary amine groups. The side-chains on derivatives I, and III are shorter than on the other derivatives.

Effect of various cholesterol derivatives on the permeability of DPPC vesicles

Table II shows the efficiency of encapsulation

TABLE II

EFFECT OF VARIOUS CHOLESTEROL DERIVATIVES ON THE LEAKAGE OF ^{59}Fe-DESFERROXAMINE ENCAPSULATED IN DPPC VESICLES

Abbreviations: CHOL, cholesterol; SA, stearylamine; PBS, phosphate-buffered saline (pH 7.4); DF, desferroxamine; 1.54:1:0.43, molar ratio of PC/Chol/SA; 1.54:0.5:0.5:0.43, molar ratio of PC/Chol/Chol derivative/SA.

Lipid composition	% Encapsulation	Leakage in	% Leakage of ^{59}Fe-DF measured by dialysis method et 37°C					
			0.5 h	1 h	2 h	4 h	19 h	24 h
Unencapsulated ^{59}Fe-DF	–	PBS	33.3	55.0	77.2	91.0	–	92.0
		serum [a]	30.0	50.0	73.2	90.4	–	93.0
DPPC/Chol/SA	3.4	PBS	4.3	7.8	14.8	18.8	–	21.4
		serum	5.6	9.7	16.2	20.2	–	22.4
DPPC/Chol/Chol-I/SA	2.7	PBS	0.3	2.4	2.2	2.5	3.1	3.5
		serum	0.4	1.6	4.3	3.7	4.0	4.8
DPPC/Chol/Chol-II/SA	4.8	PBS	1.9	2.2	2.3	4.0	4.1	–
		serum	1.2	1.8	3.0	4.4	4.3	–
DPPC/Chol/Chol-III/SA	3.3	PBS	7.2	10.8	12.5	15.5	14.6	–
		serum	14.2	31.7	48.0	58.1	55.7	–
DPPC/Chol/Chol-IV/SA	0.6	PBS	–	–	–	–	–	–
		serum	32.0	42.9	58.5	65.3	67.9	65.5
DPPC/Chol/Chol-V/SA	0.2	PBS	–	–	–	–	–	–
		serum	21.9	31.3	51.4	59.3	62.3	56.8
DPPC/Chol/Chol-VI/SA	0.4	PBS	21.9	36.9	48.0	57.0	60.9	56.5
		serum	23.5	49.3	64.0	66.7	70.2	68.9

[a] Fetal calf serum.

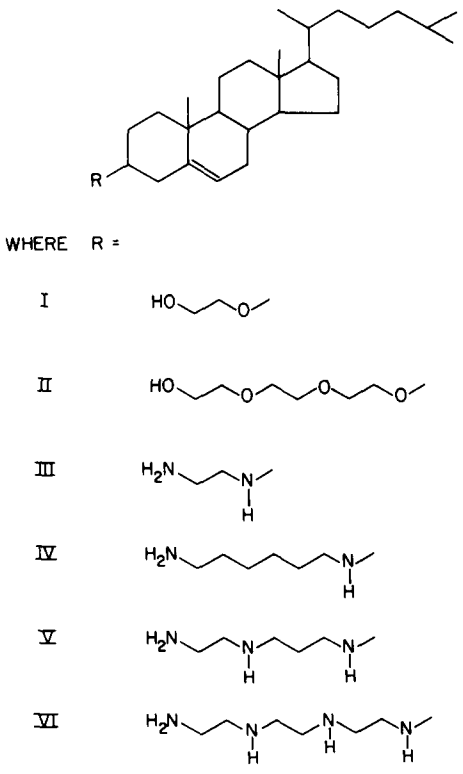

Fig. 1. Chemical structures of various synthetic cholesterol derivatives.

of the ^{59}Fe-tagged desferroxamine. Vesicles prepared in the absence of any derivative or in the presence of derivative I, II, or III were able to encapsulate about 3–5% of the total desferroxamine. Very poor encapsulation, less than 1% of the desferroxamine, resulted when derivatives IV, V, or VI were used.

Table II also shows the permeability of ^{59}Fe-tagged desferroxamine from DPPC vesicles. Unencapsulated desferroxamine is lost very rapidly from the dialysis bag (90% within 4 h). The presence of fetal calf serum has no effect on the leakage of unencapsulated desferroxamine from the dialysis bag. Encapsulation of desferroxamine in vesicles prepared from DPPC, cholesterol and stearylamine resulted in significantly less leakage of desferroxamine; thus, after 4 h only 18–20% of the encapsulated desferroxamine was lost from the vesicles. The presence of serum again had no effect on the permeability of these control vesicles.

As shown in Table II, the presence of cholesterol

derivatives I and II provides reduced permeability of vesicles with a significant reduction in the leakage of desferroxamine. Only 3–4% of encapsulated desferroxamine was lost at 4 h. Also serum had no effect on the leakage of vesicles containing cholesterol derivative I or II. The presence of cholesterol derivatives III, IV, V or VI results in very permeable vesicles, with 60% of the encapsulated desferroxamine being lost from the vesicles after only 4 h of dialysis in the presence of serum. However, vesicles prepared with derivative III were less permeable in phosphate-buffered saline, and only 15% of encapsulated desferroxamine is lost from these vesicles after 4 h of dialysis.

Effect of various cholesterol derivatives on the permeability of DSPC vesicles

Table II shows that the encapsulation of desferroxamine in vesicles containing cholesterol derivative V or VI is very low, less than 1.0%. The encapsulation in the absence of any derivative or in the presence of derivative III or IV is 1–4%.

DPPC vesicles prepared with cholesterol derivatives III, IV, V and VI were very permeable in the presence of serum. In order to reduce the permeability of vesicles, we replaced DPPC with DSPC. As shown in Table III, the permeability of vesicles prepared with DSPC, cholesterol and stearylamine was not much different from DPPC vesicles. Also, serum had no effect on the leakage of these vesicles. However, replacing DPPC with DSPC for either cholesterol derivative III- or VI-containing vesicles results in significantly reduced permeability. The rate of loss of desferroxamine for cholesterol derivative III- and VI-containing DSPC vesicles is less than half the rate of loss for the corresponding DPPC vesicles. Replacing DPPC with DSPC in cholesterol derivative IV- or V-containing vesicles has no effect on their leakage in serum. Vesicles prepared with cholesterol derivative III or IV are less permeable in phosphate-buffered saline.

Effect of cholesterol derivative II on the leakage of [^{3}H]glucose encapsulated in DPPC vesicles

The results in Table II show that replacing half of the cholesterol with cholesterol derivative II reduces the permeability of DPPC vesicles encapsulating desferroxamine. We used [^{3}H]glucose to explore these effects with DPPC vesicles with a different tracer encapsulated in the aqueous compartment. Table IV shows that 68–70% of the [^{3}H]glucose is lost from the dialysis bag after 4 h of dialysis and serum has no effect on the leakage of unencapsulated [^{3}H]glucose. When DPPC vesicles are used to encapsulate [^{3}H]glucose, their permeability in phosphate-buffered saline is similar to that seen for desferroxamine, with 17% of

TABLE III

EFFECT OF VARIOUS CHOLESTEROL DERIVATIVES ON THE LEAKAGE OF ^{59}Fe-DESFERROXAMINE ENCAPSULATED IN DSPC VESICLES

For abbreviations and molar ratios see Table II.

Lipid composition	% Encapsulation	Leakage in	% Leakage of ^{59}Fe-DF measured by dialysis method at 37°C					
			0.5 h	1 h	2 h	4 h	19 h	24 h
DSPC/Chol/SA	3.6	PBS	3.5	6.5	11.7	14.1	–	14.6
		serum [a]	3.7	6.1	11.0	13.1	–	13.5
DSPC/Chol/Chol-III/SA	2.1	PBS	5.7	10.2	13.0	14.7	16.1	17.2
		serum	10.8	15.9	19.0	21.0	21.1	22.5
DSPC/Chol/Chol-IV/SA	1.2	PBS	5.9	8.6	9.4	11.7	14.3	15.2
		serum	28.9	41.6	54.4	58.8	58.5	59.6
DSPC/Chol/Chol-V/SA	0.3	PBS	–	–	–	–	–	–
		serum	28.5	38.8	39.9	50.3	51.3	53.5
DSPC/Chol/Chol-VI/SA	0.2	PBS	–	–	–	–	–	–
		serum	8.0	8.9	10.4	9.3	12.7	14.1

[a] Fetal calf serum.

TABLE IV

EFFECT OF CHOLESTEROL DERIVATIVE II ON THE LEAKAGE OF [^{3}H]GLUCOSE ENCAPSULATED IN DPPC VESICLES

For abbreviations and molar ratios see Table II.

Lipid composition	% Encapsulation	Leakage in	% Leakage of [^{3}H]glucose measured by dialysis method at 37°C					
			0.5 h	1 h	2 h	4 h	19 h	24 h
Unencapsulated [^{3}H]glucose	–	PBS	44.6	63.8	68.5	67.8	65.4	67.0
		serum [a]	47.4	63.2	69.8	69.1	66.7	69.2
DPPC/Chol/SA	4.1	PBS	4.6	6.9	12.8	17.4	29.4	33.3
		serum	9.1	18.4	32.9	43.4	52.4	53.5
DPPC/Chol/Chol-II/SA	2.9	PBS	4.0	5.4	8.9	10.5	21.5	22.8
		serum	3.3	5.7	9.4	11.1	20.6	22.1

[a] Fetal calf serum.

the encapsulated [^{3}H]glucose being lost after 4 h of dialysis. However, when these vesicles are incubated in serum, glucose leakage is 2–2.5-times higher than in phosphate-buffered saline. When half of the cholesterol is replaced with a cholesterol derivative II, the permeability is reduced, with vesicles being equally permeable in both phosphate-buffered saline and serum, and only 11% of the glucose leaking out after 4 h.

Effect of derivative II or III on the tissue distribution of vesicles in vivo

Table V shows the tissue distribution of unencapsulated and liposome-encapsulated ^{59}Fe-tagged desferroxamine in mice. Unencapsulated desferroxamine is removed from the body very rapidly and only 13.1% of the injected dose is recovered from blood, bone marrow, heart, lung, liver, small and large intestine, spleen, kidneys, stomach and

TABLE V

MOUSE TISSUE DISTRIBUTION AT 2 h AFTER INTRAVENOUS INJECTION OF UNENCAPSULATED AND PHOSPHOLIPID-VESICLE-ENCAPSULATED ^{59}Fe-DESFERROXAMINE

Each value represents an average of four mice ± the standard error of the mean. For abbreviations and molar ratios see Table II.

Tissue	% Injected dose of vesicles			
	Unencapsulated	DSPC/Chol/SA	DSPC/Chol/Chol-II/SA	DSPC/Chol/Chol-III/SA
Blood [a]	3.0 ± 0.6	54.6 ± 1.4	43.3 ± 2.3	4.7 ± 0.9
Bone marrow [b]	4.7 ± 0.9	7.9 ± 0.4	8.9 ± 1.3	5.7 ± 1.2
Heart	0.1 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.1 ± 0.0
Lung	0.1 ± 0.0	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.1
Liver	1.5 ± 0.2	17.3 ± 1.1	18.4 ± 0.4	41.3 ± 2.0
Small intestine [c]	1.1 ± 0.2	3.5 ± 0.4	2.9 ± 0.2	1.3 ± 0.2
Kidney	0.9 ± 0.1	2.1 ± 0.3	1.8 ± 0.2	0.8 ± 0.2
Spleen	0.9 ± 0.3	1.7 ± 0.3	1.5 ± 0.1	2.0 ± 0.2
Brain	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Stomach [c]	0.1 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	–
Large intestine [c]	0.9 ± 0.2	1.5 ± 0.3	2.1 ± 0.4	–
% Recovery	13.1 ± 4.7	90.4 ± 3.0	80.7 ± 3.6	56.5 ± 3.2

[a] The amount of radioactivity in total blood was obtained by presuming that blood comprises 7.3% of the total weight of the animals [17].

[b] The amount of radioactivity in total marrow was obtained by multiplying the amount of radioactivity measured in the marrow of a pair of tibias by the factor of 44 [22].

[c] Values are calculated including internal contents.

brain. However, encapsulation of desferroxamine in vesicles results in 60–90% recovery of desferroxamine from the above organs. At 2 h after intravenous injection, 54.5% of the injected dose of vesicles was still circulating in the blood, and 7.9 and 17.3% of the injected dose of vesicles was recovered from bone marrow and liver, respectively. Replacing half the cholesterol with cholesterol II reduces the permeability of vesicles (Tables II and IV). However, Table V shows that cholesterol II has no effect on the tissue distribution of vesicles. When half of the cholesterol is replaced with derivative III, three major differences in tissue distribution are observed. The total recovery of encapsulated desferroxamine in cholesterol III-containing vesicles is only 56.5% among all these organs. Also, cholesterol III-containing vesicles are removed very rapidly from the blood, with only 4.7% of the initial dose in the blood after 2 h. This is accompanied by significantly higher liver uptake.

Discussion

Our results show that vesicles encapsulating desferroxamine prepared either with DPPC or DSPC are equally permeable in phosphate-buffered saline or bovine serum. The permeability of vesicles containing DSPC appears to be slightly less than that of DPPC-containing vesicles. Mauk and Gamble [18] have reported similar results using the γ-ray-perturbed angular correlation technique. Replacing half of the cholesterol with synthetic derivatives having a hydroxyl terminal group on the side-chain, (I and II) reduces the permeability of DPPC vesicles. However, addition of synthetic derivatives with terminal amino groups (III, IV, V and VI) increases the permeability of DPPC vesicles. The four amino-containing derivatives also caused marked permeability differences. This behavior appears to correlate with the length of the groups separating the terminal amine from the sterol nucleus. Thus, the ten-bond derivative VI in DPPC vesicles was very permeable in both phosphate-buffered saline and serum, encapsulating only 0.4% of the desferroxamine, while the shortest (four-bonds) derivative III encapsulated 3.3% and was as permeable as the control vesicles in phosphate-buffered saline, although markedly permeable in serum. The two eight-bond derivatives (IV and V) behaved similarly to the ten-bond derivative VI. The poor encapsulation and higher permeability of the longer derivatives may relate to either steric effects or charge interactions with the phospholipids [23–26] or serum proteins [27–30]. The greater spacer length of these derivatives could result in longer range, more stable electrostatic interactions among neighboring lipid phosphate groups and the amino-cholesterol moiety. Such effects could influence the lipid distribution related to volume effects and inside/outside ratios, possibly introducing instability through defect formation [31,32] or perturbations in bilayer elasticity [33]. The decrease in phosphate-buffered saline permeability and increase in the volume of encapsulation seen for the short-chain derivative III could be the result of weaker electrostatic interactions, allowing the lipids greater motional freedom to form a more stable bilayer structure. The higher permeability of derivative III-containing vesicles in serum is overcome when the higher melting DSPC is used (see Table III). This result may be related to an increase in vesicle size [34] thus reducing strain, or a decrease in fluidity which has been shown to modulate vesicle-protein interactions such as vesicle lysis by the serum-complement system [35].

The marked reduction in vesicle permeability seen when hydroxylated cholesterol analogs are used may be due to improved steric interactions at the vesicle surface. A role for lipid shape in determining membrane structure has long been considered important [36,37]. The larger, hydrophilic 'headgroups' of derivative I and II may provide a better balance than cholesterol for interacting with the phospholipids in the highly curved vesicle outer surface [38]. In fact, derivative II alone has been found to form stable membranes in aqueous solutions [20]. Consistent with this explanation is the observation that the reduced permeability is independent of the medium (either phosphate-buffered saline or serum), as would be expected for vesicles intrinsically stabilized by better lipid packing. It is interesting that the leakage of glucose was found to be more rapid than desferroxamine, for both control and derivative II-containing vesicles (Table IV). The most likely explanation for this observation is that the reduced size and charge neutrality

of glucose, relative to desferroxamine, allows the former to pass through the bilayer more easily. The large difference in leakage between phosphate-buffered saline and serum for the control vesicles indicates some additional perturbation induced by the serum proteins, while for the stabilized derivative II-containing vesicles the increased leakage is the same in both media, consistant with the physical differences between glucose and desferroxamine.

The tissue distribution results indicate that desferroxamine injected by the intravenous route is removed rapidly from the body. However, within the same time period, most of the vesicle-encapsulated desferroxamine is recovered. The presence of derivative II has no effect on the tissue distribution of vesicles. However, the presence of derivative III, the four-bond amine-containing derivative, drastically alters the tissue distribution of vesicles. These vesicles are cleared from the blood much faster than control vesicles. Such a rapid blood clearance is accompanied by a higher liver uptake. Mauk et al. [17] have reported that amino-mannose-containing vesicles are rapidly cleared from the bloodstream with higher liver uptake. Wu et al. [15] later demonstrated that the inclusion of aminomannose on the surface of liposomes causes a high rate of phagocytosis by macrophages. It is possible that the more cationic derivative III-containing vesicles are also taken up more rapidly by phagocytic cells.

The lack of effect that derivative II-containing vesicles have on the tissue distribution and rate of blood clearance is consistent with the in vitro observation that this derivative reduces the permeability of the vesicle. The practical development of vesicles for in vivo drug delivery requires that stable formulations be developed in order to provide sufficient shelf-life for such systems to be economically feasible. The study of molecules, such as derivative II, which intrinsically stabilize vesicles without affecting their in vivo tissue distribution, should prove useful in complementing the development of formulations for targeted vesicle systems [17,39].

Acknowledgments

We wish to thank Dr. Robert Ireland for the use of the JASCO-DIP-181 digital polarimeter and Dr. Robert Kaiser of the Southern California Regional NMR Facility, California Institute of Technology, Pasadena, CA for assistance in obtaining proton nuclear magnetic resonance spectra. This research was supported by grants from the National Institutes of Health (GM-21111-11), the National Science Foundation (CHE81-12589), and the Monsanto Company. This is contribution No. 7037 from the Arthur Amos Noyes Laboratory of Chemical Physics.

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