Drug Development and Industrial Pharmacy, 22(3), 217–224 (1996)

Application of ^{99m}Tc Labeling for Fast Comparative Screening of Preformed Liposomes In Vivo

Zoi Panagi,[1] Christine Sawas-Dimopoulou,[1] Konstantinos Avgoustakis,[2] and Dionyssis S. Ithakissios*,[1,2]

[1]*Institute of Radioisotopes and Radiodiagnostic Products, NCSR Demokritos, Aghia Paraskevi Attikis, Athens 153 10, Greece*
[2]*Pharmaceutical Technology Lab, University of Patras, Rion 261 10, Greece*

ABSTRACT

Labeling of preformed liposomes with ^{99m}Tc is a simple, quick, and highly versatile method, and its potential in the comparative screening of preformed liposomes with regard to their early biodistribution properties, such as the BLOOD/RES ratios at 2 and 30 min postadministration, was investigated. Liposomes differing in lipid composition and size were prepared and labeled under optimum conditions with ^{99m}Tc using a slight modification of a previously described method (1). The amount of $SnCl_2$ used to reduce pertechnetate affected liposome biodistribution. The labeling method employed was capable of detecting anticipated changes in liposome biodistribution caused by alterations in liposome composition or size. It could also reveal the effect of a relative immiscibility of monosialganglioside GM_1 with di-myristoyl-phosphatidyl-choline:di-myristoyl-phosphatidyl-glycerol (DMPC:DMPG) on the biodistribution of DMPC:DMPG:GM_1 liposomes. It is proposed that ^{99m}Tc labeling of liposomes provides a tool for fast comparative screening of preformed liposomal preparations according to their early biodistribution.

*To whom correspondence should be addressed: Institute of Radioisotopes and Radiodiagnostic Products, NCSR Demokritos, Aghia Paraskevi Attikis, Athens 153 10, Greece.

INTRODUCTION

Liposomes have attracted considerable interest for applications in the areas of diagnostics, drug delivery, genetic engineering, and immunomodulation. They are biocompatible, biodegradable, and normally non-immunogenic lipid vesicles enclosing aqueous and/or lipid phases, protecting them from enzymatic degradation and other inactivation processes. Systemically injected liposomes are usually rapidly and efficiently taken up by phagocytic cells of the reticuloendothelial system (RES), and this has been a major obstacle in targeting liposomes and delivering drugs to other than the RES tissues. On the other hand, in the last few years the interest of using liposomes as a targeting system has increased through the utilization of selected lipid compositions and membrane modifications that prolong blood vesicle circulation and improve target specificity.

Labeling of liposomes with radionuclides can facilitate the screening of new formulations providing useful information on their in vivo kinetics. On the other hand, so far, there is no universal labeling method available for liposomes. External ^{99m}Tc labeling of liposomes (1) can be applied to a broad range of lipid compositions but suffers with regard to its biostability (2). Therefore, in the present study, we investigated the potential of using the method of ^{99m}Tc labeling of preformed liposomes as a tool for comparative screening of early biodistribution properties of various liposomal formulations. Two basic liposomal compositions were studied: DMPC:DMPG (7:3) and DSPC:CH (2:1), and these were modified with respect to lipid composition and size in order to bring about predictable changes in liposome biodistribution. The ability of labeling with ^{99m}Tc to reveal these changes in vivo was then evaluated.

MATERIALS AND METHODS

Di-myristoyl-phosphatidyl-glycerol (DMPG), distearoyl-phosphatidyl-choline (DSPC), cholesterol (CH), and bovine brain monosialganglioside (GM_1) were purchased from Sigma (St.Louis, MO). Polyethyleneglycol-distearoylphosphatidyl-ethanolamine (PEG-DSPE), molecular weight ca. 2691, was obtained from Genzyme (Haverhill, Suffolk, England). Carrier-free ^{99m}Tc was obtained from NCSR Demokritos (Athens, Greece). Bovine serum albumin was from Riedel-de Haen (Hannover). Miscellaneous reagents were obtained from Sigma (St. Louis), and solvents, all of analytical grade, were from Farmitalia Carlo Erba (Milano, Italy) or Riedel-de Haen (Hannover).

Liposome Preparation

Multilamellar liposomes (MLV) were prepared using 12–13 μmol lipids/ml by the lipid film hydration method (3) and downsized by extrusion through polycarbonate membranes (Millipore filter type: TMTP) of 5 μ pore size. Vesicle size was evaluated using a Leitz optical microscope coupled with a Philips solid-state CCD camera. Microscopic images were processed with the FG-100 card of Image Processing IWC. The software used was from Media Cybernetics Inc. MLV size ranged from 0.3 μ to 6.5 μ with mean diameter 2.0 ± 0.5 μ. Unilamellar liposomes (LUV) with homogeneous size distribution were obtained by repeatedly extruding MLV preparations through polycarbonate membranes (Avestin, Canada) of 0.1 μ pore size using a Liposofast apparatus (4). The lipid molar ratios for both MLV and LUV liposomes were: DMPC:DMPG (7:3), DMPC:DMPG:GM_1 (7:3:0.25), DMPC:DMPG:GM_1 (7:3:0.5), DMPC:DMPG:GM_1 (7:3:1), DMPC:DMPG:DSPE-PEG (7:3:1), DSPC:CH (2:1), DSPC:CH:GM_1 (2:1:0.33), DSPC:CH:DSPE-PEG (2:1:0.33). The DSPE-PEG lipid fraction, which did not incorporate into the liposomes, was removed by passing the vesicles through Sephadex G50 columns. The PEG content of liposomes was determined using the Bradford method (5).

In Vitro Stability of Liposomes

The stability of vesicles can be evaluated from their resistance against ethanol (6). In this method, small aliquots of ethanol are successively added in a vesicle dispersion and the absorbance of the dispersion at 450 nm is plotted against the ethanol content (v/v) of the dispersion. The higher the slope of such a plot, the lower the stability of the dispersion. This method was adapted here to evaluate the stability of certain liposomal formulations. Thus, 0.025 ml aliquots of absolute ethanol were added to 1 ml of liposome suspension containing 2.5 μmol lipids/ml, and the absorbance change at 450 nm was measured using a DMS 90 VARIAN spectrophotometer. The observed absorbance (A_{obs}) was corrected to compensate for dilution using the following equation:

$$A_{obs} = A_{corr} \times \frac{(\text{ml of liposomes} + \text{ml of alcohol})}{(\text{ml of liposomes})}$$

Liposome Thermal Behavior

To assess the degree of mixing of GM_1 with DMPC:DMPG (7:3) in the smectic mesophase state, differential scanning calorimetry (DSC) was conducted on DMPC:DMPG:GM_1 liposome samples containing about 20 mg total lipid. The GM_1/DMPC:DMPG molar ratios were 0:100, 5:95, and 10:90. The samples were centrifuged at 21,000 rpm for 30 min and the liposome pellets were transferred to the sample cell of a R90 Perkin Elmer instrument. Thermograms in the range of 0–40°C were obtained at a scan rate of 5°C in the high-sensitivity mode.

^{99m}Tc Labeling Procedure

The technetium-labeling procedure for preformed liposomes was based on the method proposed by Richardson et al. (1) with slight modifications. Briefly, a sterile solution of $SnCl_2$ (3 μM) was prepared in oxygen-free saline (0.9% NaCl in water), kept under nitrogen, and 0.5 ml from this solution was immediately mixed with 1 ml of liposomes followed by the addition of 0.5 ml (6–10 mCi) $Na^{99m}TcO_4$ solution in saline. The mixture was vigorously shaken and was considered ready to be used after 15 min incubation at room temperature. Purification of the labeled product was not necessary since labeling efficiency was always higher than 98% as determined by paper chromatography in Whatman No. 1 paper and phosphate buffer pH 7.4 as eluent. The effect of the amount of $SnCl_2$ on liposome properties was examined by changing the concentration of the $SnCl_2$ solution added in liposomes.

In Vitro Stability of Labeling

The stability of ^{99m}Tc association with liposomes was determined by measuring the radioactivity still bound on liposomes after 2, 30, and 60 min incubation at 37°C in saline or in saline-diluted mice serum (1:1 v/v). Liposome quantities of 0.1 ml were added in 0.2 ml incubation medium, and at the predetermined time intervals, aliquots of the above mixtures were spotted on Whatman No. 1 paper for development in a 0.1 M acetate buffer pH 5.8 eluant. Radioactivity bound to liposomes remained at the origin, while all soluble forms of it migrated. Control studies were carried out with albumin and $Na^{99m}TcO_4$. Albumin was visualized with iodine and it was found to migrate to the top of the chromatograms whereas only 0.2% of free $Na^{99m}TcO_4$ remained at the origin.

In Vivo Studies

The tissue distribution of labeled liposomes was determined in Swiss/De male mice, 3 months old, weighing 20–30 g. Prior to liposome administration the animals were allowed free access to food and tap water. Animals were injected at random in the tail vein with 0.1 ml liposome suspension containing 0.60–0.70 μmol lipids (6–8 mCi), and transferred onto paper filters, which would absorb animals' urine, trapping them under reversed glass beakers. The animals were sacrificed by decapitation with a guillotine 2 and 30 min after injection. Blood samples were obtained by cardiac puncture in preweighed heparinized tubes and tissues were excised, washed quickly with cold water to remove surface blood, weighed, and then counted for radioactivity. The label excreted in urine was calculated by adding the radioactivity of urinary bladder to the radioactivity of the paper filter used to collect animal urine. Each experiment was replicated three times. Data were normalized and corrected for the decay of the isotope over the period of measurement. Blood and muscles were taken to weigh 7% and 43% of body weight respectively.

RESULTS

Effect of Stannous Chloride Concentration

The effect of $SnCl_2$/lipid weight ratio on the early biodistribution of ^{99m}Tc-DSPC:CH (2:1) LUVs was tested 2 min after liposome administration in mice (Figure 1). For the time tested the $SnCl_2$/lipid ratio did not appear to affect radioactivity excretion; however, it had a significant effect on radioactivity levels in other tissues. Increasing the $SnCl_2$/lipid ratios from 0.004 to 0.03 tended to increase the blood level and decreased significantly ($P < 0.05$) RES uptake of liposomes. A further increase of $SnCl_2$/lipid ratio to 0.3 had the opposite effect, i.e. it significantly ($P < 0.05$) decreased blood level and slightly increased RES uptake. Also, there was a significant increase ($P < 0.05$) in muscle radioactivity. Similar results were also obtained with DMPC:DMPG (7:3) liposomes (data not shown).

In Vitro Stability of Labeling

Following the labeling protocol described in the Materials and Methods section, more than 98% of ^{99m}Tc added was bound on liposomes. Label loss, after 1 h incubation of radiolabeled vesicles at 37°C, was less

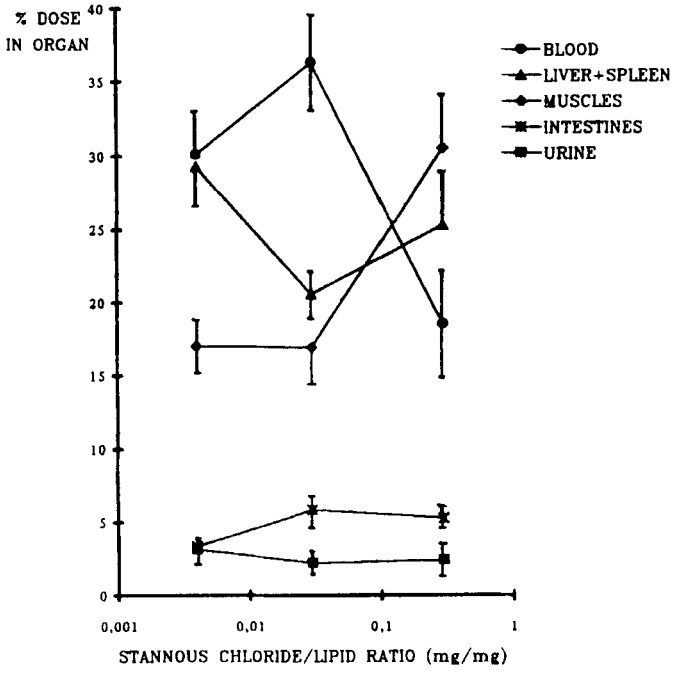

Figure 1. Effect of $SnCl_2$/lipid ratio on the biodistribution of DSPC:CH (2:1) liposomes, 2 min after intravenous injection in mice.

than 4% in 0.9% NaCl solution (saline) and less than 13% in diluted mice serum, respectively (Table 1).

In Vitro Stability of Liposomes

The stability of liposomes was investigated by recording the absorbance change of the liposomal dispersions caused by the succesive addition of ethanol, as described in Materials and Methods. Least square fits of the lipid absorbance versus ethanol content (% v/v) data were obtained for DMPC:DMPG liposomes containing different amounts of GM_1, and the slopes of these fits are shown in Table 2. The higher the slope, the lower the stability of liposomes. The stability of DMPC:DMPG liposomes slightly, but consistently, decreased as their content in GM_1 increased.

Table 1

^{99m}Tc Label Remaining on DMPC:DMPG Liposomes After Incubation in Saline and in Mice Serum at 37°C

Time (min)	Saline	Serum
2	99.66 ± 1.18[a]	97.56 ± 7.32
30	98.38 ± 2.04	90.99 ± 3.23
60	96.17 ± 1.93	87.30 ± 3.86

[a]% initial radioactivity S.D.

Thermal Behavior of Liposomes

The miscibility of GM_1 with DMPC:DMPG in the smectic mesophase state was investigated by calorimetry. The addition of GM_1 into the DMPC:DMPG bilayers had two consequences in the melting behavior of DMPC:DMPG liposomes. It increased the melting point slightly and broadened the peak of the transition significantly (Figure 2).

Biodistribution of ^{99m}Tc-Labeled Liposomes

The biodistribution of radioactivity associated with liposomes was determined 2 and 30 min postadministration in mice. The incorporation of appropriate amounts of GM_1 or PEG-DSPE in the basic lipo-

Table 2

Slopes of the Least Square Fits (and the Respective Correlation Coefficients) for the Absorbance of the Liposome Dispersion Versus the Ethanol Added (% v/v) Data of DMPC:DMPG and DMPC:DMPG:GM1 Liposomes

Liposome Composition (molar ratio)	Slope	Correlation Coefficient
DMPC:DMPG (7:3)	-0.051	-0.9970
DMPC:DMPG:GM1 (7:3:0.25)	-0.055	-0.9988
DMPC:DMPG:GM1 (7:3:0.50)	-0.057	-0.9982
DMPC:DMPG:GM1 (7:3:1)	-0.066	-0.9972

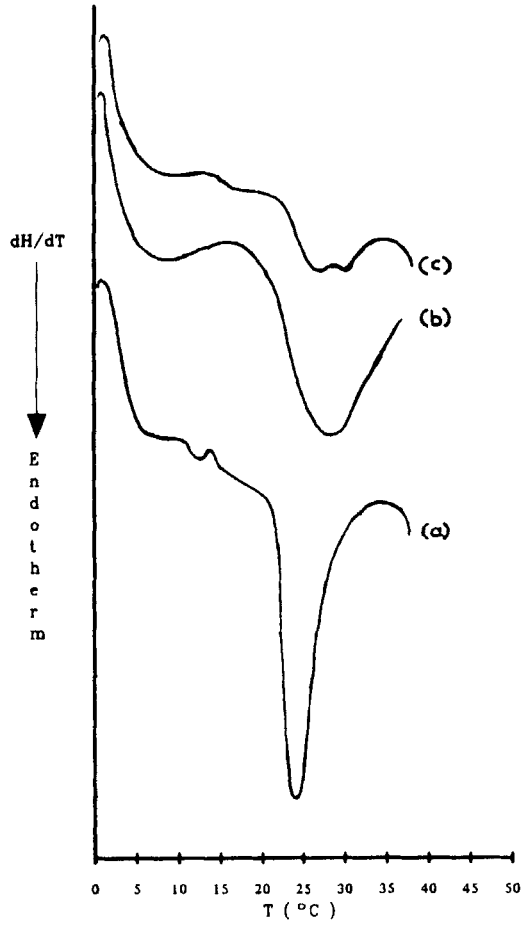

Figure 2. Thermal analysis of DMPC:DMPG liposomes: (a) DMPC:DMPG (7:3), (b) DMPC:DMPG:GM_1 (7:3:0.5), (c) DMPC:DMPG:GM_1 (7:3:1).

somal compositions DMPC:DMPG(7:3) and DSPC:CH(2:1) caused a significant increase in the BLOOD/RES ratio in both MLV and LUV liposomes (Tables 3 and 4).

Increasing GM_1 molar ratio in DMPC:DMPG:GM_1 formulations beyond 0.25 (corresponding to 2.24% mol GM_1 in the formulation) decreased the BLOOD/RES ratios (Table 5).

In liposomes having the same lipid composition the reduction of size from MLV to LUV caused a significant increase in the BLOOD/RES ratios of liposomes, with the exception of liposomes containing PEG-DSPE, whose BLOOD/RES ratios at 2 min postinjection decreased upon reduction of their size (Tables 3 and 4).

DISCUSSION

Radiolabeling of liposomes with γ-emitting radionuclides facilitates the study of liposome in vivo properties. In this work ^{99m}Tc was selected as a tracer due to its high availability and its excellent nuclear properties. Several methods of liposome labeling with ^{99m}Tc have appeared in the literature, which usually involve the encapsulation of water-soluble chelating agents in the aqueous phase or lipid derivatives of chelators in the lipid phase of liposomes, in order to efficiently entrap ^{99m}Tc in liposomes (7–10). The need for tailor-made chelating agents, the low encapsulation efficiency and therefore the need for a purification step, and the possibility of the chelating agent affecting liposome properties, as previous relative research has shown (8,10), are the major drawbacks of this approach. To our knowledge, the only method that could be applied to any type of preformed liposomes is that proposed by Richardson et al. (1). On the other hand, ^{99m}Tc labeling of preformed liposomes has found limited use so far due to low stability in vivo (2). Nevertheless, because of labeling simplicity, rapidity, and versatility, we investigated the possibility of applying this method to screen, rapidly and in a comparative sense, liposomal formulations according to their early biodistribution properties.

We proposed optimum ^{99m}Tc-labeling conditions for liposomes, selected by studying the effect of the amount of $SnCl_2$, used to reduce ^{99m}Tc, on the biodistribution of ^{99m}Tc-labeled DSPC:CH (2:1) LUV liposomes. The highest BLOOD/RES ratio was obtained at 0.03 $SnCl_2$/lipid ratio. Increasing $SnCl_2$/lipid ratio beyond 0.03 resulted in a significant decrease of radioactivity in blood with a subsequent increase primarily in muscles, but also in RES (Figure 1). The reasons for radioactivity accumulation in muscles are not well understood and need further investigation, but the accumulation of radioactivity in RES may be due to the aggregation of vesicles in the presence of excess quantities of Sn^{++}.

We observed that in using $SnCl_2$/lipid ratios as high as those employed by Richardson et al. (1), i.e., 0.14 and higher, liposomes aggregated in vitro, forming a gel-like structure within 24 h storage. Some reasons for this aggregation, which occurred not only with DSPC:CH but also with DMPC:DMPG and other liposomal formulations, may be the formation of colloid stannous hydroxides, or liposome flocculation via a mechanism similar to that operating when Ca^{++} are

Table 3

Distribution of MLVs in Blood and RES, 2 and 30 min Postadministration, in Mice

Liposome Composition (molar ratio)	Blood	RES		BLOOD/RES
		Liver	Spleen	
2 min				
DMPC:DMPG (7:3)	23.33 ± 1.99[a]	45.19 ± 5.67	1.65 ± 0.72	0.507 ± 0.101
DMPC:DMPG:GM1 (7:3:0.25)	25.56 ± 2.87	20.32 ± 3.86	0.53 ± 0.08	1.359 ± 0.198
DMPC:DMPG:PEG-DSPE (7:3:1)	52.15 ± 1.30	13.85 ± 1.17	0.57 ± 0.13	3.631 ± 0.333
DSPC:CH (2:1)	19.75 ± 6.78	53.61 ± 9.40	2.19 ± 0.39	0.372 ± 0.169
DSPC:CH:GM1 (2:1:0.33)	29.39 ± 7.82	28.32 ± 4.37	3.11 ± 1.66	0.985 ± 0.407
DSPC:CH:PEG-DSPE (2:1:0.33)	53.27 ± 1.87	14.75 ± 0.23	0.50 ± 0.07	3.498 ± 0.109
30 min				
DMPC:DMPG (7:3)	13.84 ± 2.24	42.84 ± 5.89	2.63 ± 0.81	0.310 ± 0.079
DMPC:DMPG:GM1 (7:3:0.25)	11.48 ± 2.89	19.80 ± 0.41	0.88 ± 0.38	0.500 ± 0.146
DMPC:DMPG:PEG-DSPE (7:3:1)	20.81 ± 2.00	21.69 ± 1.50	1.08 ± 0.13	0.921 ± 0.144
DSPC:CH (2:1)	6.31 ± 1.63	54.92 ± 1.03	1.27 ± 0.31	0.113 ± 0.031
DSPC:CH:GM1 (2:1:0.33)	9.84 ± 2.21	36.47 ± 5.36	5.47 ± 0.66	0.233 ± 0.027
DSPC:CH:PEG-DSPE (2:1:0.33)	16.04 ± 0.51	27.28 ± 2.26	1.03 ± 0.12	0.567 ± 0.032

[a]% injected dose ± S.D.

added in liposomes containing acidic phospholipids (3). A 0.03 $SnCl_2$/lipid ratio, which gave the highest blood levels and lowest RES uptake of DSPC:CH liposomes, was selected for the rest of this work. This ratio is five times lower than that proposed by Richardson et al. (1).

In order to investigate the potential of ^{99m}Tc labeling as a tool for relative in vivo screening of liposomal formulations, we studied two basic lipid compositions, DSPC:CH (2:1) and DMPC:DMPG (7:3), which have been shown to form stable liposomes and have been

Table 4

Distribution of LUVs in Blood and RES, 2 and 30 min Postadministration, in Mice

Liposome Composition (molar ratio)	Blood	RES		BLOOD/RES
		Liver	Spleen	
2 min				
DMPC:DMPG (7:3)	37.76 ± 1.33[a]	29.16 ± 1.98	1.33 ± 0.05	1.022 ± 0.375
DMPC:DMPG:GM1 (7:3:0.25)	45.30 ± 3.35	22.32 ± 0.80	2.25 ± 0.30	1.921 ± 0.216
DMPC:DMPG:PEG-DSPE (7:3:1)	56.07 ± 2.34	16.16 ± 2.07	0.69 ± 0.11	3.375 ± 0.592
DSPC:CH (2:1)	35.44 ± 3.52	18.85 ± 1.47	0.63 ± 0.09	1.840 ± 0.337
DSPC:CH:GM1 (2:1:0.33)	41.29 ± 0.82	10.59 ± 1.41	0.53 ± 0.04	3.758 ± 0.522
DSPC:CH:PEG-DSPE (2:1:0.33)	45.87 ± 3.84	18.67 ± 2.21	0.91 ± 0.42	2.638 ± 0.261
30 min				
DMPC:DMPG (7:3)	17.96 ± 5.79	39.55 ± 1.43	2.55 ± 0.75	0.339 ± 0.043
DMPC:DMPG:GM1 (7:3:0.25)	10.71 ± 1.12	7.85 ± 0.22	0.33 ± 0.06	1.305 ± 0.092
DMPC:DMPG:PEG-DSPE (7:3:1)	24.69 ± 1.27	22.03 ± 1.16	1.37 ± 0.21	1.057 ± 0.054
DSPC:CH (2:1)	13.18 ± 1.28	22.75 ± 1.51	0.68 ± 0.19	0.560 ± 0.076
DSPC:CH:GM1 (2:1:0.33)	18.46 ± 2.58	17.14 ± 2.30	1.01 ± 0.08	1.145 ± 0.140
DSPC:CH:PEG-DSPE (2:1:0.33)	21.43 ± 3.12	20.74 ± 1.51	1.18 ± 0.37	0.860 ± 0.140

[a]% injected dose ± S.D.

Table 5

Biodistribution of DMPC:DMPG Liposomes with Different GM_1 Content

Liposome Composition (molar ratio)	Type	Blood	RES		BLOOD/RES
			Liver	Spleen	
2 min					
DMPC:DMPG :GM1(7:3:0.25)	MLV	25.56 ± 2.87[a]	20.32 ± 3.86	0.53 ± 0.08	1.359 ± 0.196
	LUV	45.30 ± 3.35	22.32 ± 0.80	2.25 ± 0.30	1.921 ± 0.216
DMPC:DMPG:GM1(7:3:0.5)	MLV	14.67 ± 4.56	68.53 ± 4.65	2.06 ± 0.32	0.211 ± 0.082
	LUV	31.39 ± 1.23	50.52 ± 0.70	1.69 ± 0.64	0.601 ± 0.023
DMPC:DMPG:GM1 (7:3:1)	MLV	23.73 ± 2.53	54.86 ± 2.13	1.91 ± 0.82	0.419 ± 0.066
	LUV	43.17 ± 2.34	30.56 ± 3.31	1.59 ± 0.47	1.160 ± 0.205
30 min					
DMPC:DMPG :GM1(7:3:0.25)	MLV	11.48 ± 2.89	19.80 ± 0.41	0.88 ± 0.38	0.500 ± 0.147
	LUV	10.71 ± 1.12	7.85 ± 0.22	0.33 ± 0.06	1.305 ± 0.092
DMPC:DMPG:GM1(7:3:0.5)	MLV	12.43 ± 0.78	64.31 ± 2.76	2.18 ± 0.10	0.173 ± 0.040
	LUV	5.78 ± 0.57	65.44 ± 0.81	2.81 ± 0.73	0.085 ± 0.009
DMPC:DMPG:GM1 (7:3:1)	MLV	9.63 ± 1.85	60.41 ± 3.27	2.80 ± 0.52	0.153 ± 0.022
	LUV	14.66 ± 1.00	33.91 ± 1.67	1.81 ± 0.60	0.409 ± 0.014

[a]% injected dose ± S.D.

considered for the delivery of antifungal and anticancer agents (11,12). Modifications were introduced into the above basic formulations in order to cause predictable changes in liposome biodistribution, and then the ability of ^{99m}Tc labeling to reveal these changes was evaluated. The modifications were affected by adding GM_1 or PEG-DSPE into the basic lipid compositions. The incorporation of GM_1 or PEG-DSPE, which have bulky and hydrophilic polar regions, in liposome bilayers has been shown to significantly prolong the circulation of liposomes in blood and supress their uptake by RES (13,14). These effects are more pronounced in the case of the polymer-derivatized lipid PEG-DSPE (14).

Biodistribution studies of both the basic and the modified liposomes were performed in mice. The addition of appropriate amounts of GM_1 or PEG-DSPE in both basic compositions resulted in increased BLOOD/RES ratios for both MLV and LUV liposomes (Tables 3 and 4). Also, the reduction of liposome size, from MLV to LUV, provided increased BLOOD/RES ratios (Tables 3 and 4). These results are entirely consistent with the literature (14–17). An exception was the results obtained at 2 min postinjection with PEG-DSPE containing liposomes, in which the reduction of size decreased BLOOD/RES ratios (Tables 3 and 4). This may be attributed to the partial loss of active PEG molecules during the manipulations for reduction of liposome size.

It may be postulated that in LUV liposomes PEG-DSPE molecules are equally distributed between the inner and the outer side of the bilayer, whereas in MLV liposomes, in which the space between the bilayers is limited, the PEG-DSPE could preferentially be positioned in the outer side of the surface bilayer, due to the bulkiness of PEG-DSPE molecules. Therefore, the breaking and reconstitution of liposome membranes during LUV formation through MLV extrusion may bring more PEG-DSPE molecules in the inner half of the bilayers. This assumption was supported by the results of the Bradford method, with which only the PEG on the liposome surface can be assayed. Thus, for DMPC:DMPG:PEG-DSPE liposomes the PEG-content was 6.06% mol and 3.05% mol, and for DSPC:CH:PEG-DSPE it was 6.93% mol and 3.58% mol before and after the extrusions, respectively. The reduction of PEG concentration on the liposome surface lowers the protection that PEG provides to liposomes against blood constituents, and probably accounts for the decrease in the BLOOD/RES ratio at 2 min postinjection observed upon reduction of the size of PEG containing liposomes. The reduced PEG content of LUVs, as compared to MLVs, probably had a more significant effect at the early stage of liposome interaction with the biological system, i.e., at 2 min postinjection, since PEG containing LUVs had the same or slightly higher BLOOD/RES

ratios than the respective MLVs at 30 min postinjection (Tables 3 and 4).

The incorporation of 2.24% mol GM_1 into DMPC:DMPG bilayers, formulation DMPC:DMPG:GM_1 (7:3:0.25), resulted in increased BLOOD/RES ratios that may be attributed to the increased hydrophilicity of DMPC:DMPG:GM_1 liposomes due to the presence of GM_1 (Tables 3 and 4). With a further increase of GM_1 content, the BLOOD/RES ratio decreased (Table 5). In an attempt to elucidate these conflicting at first sight results, we examined first the stability of liposome membranes in the presence of increased amounts of GM_1 using the ethanol test, and second the miscibility of GM_1 with the host bilayer by DSC. Increasing GM_1 concentration into DMPC:DMPG bilayers reduced the stability of liposomes (Table 2). This was attributed to a failure of GM_1 to uniformly be dispersed in the host bilayer. The results from the thermal analysis supported this assumption, since the addition of GM_1 into DMPC:DMPG slightly increased the melting point and significantly broadened the peak of the transition, indicating a relative immiscibility of the above lipids (Figure 2) that may originate from their structural differences. Reduced stability may be the reason for the decreased BLOOD/RES ratios observed when more than 2.24% mol GM_1 was incorporated in DMPC:DMPG liposomes (Table 5).

In this work, we proposed optimum conditions for ^{99m}Tc labeling of preformed liposomes. We found that ^{99m}Tc-labeling of liposomes was sufficiently stable in vitro (mice serum) for at least 1 hr. We also found that ^{99m}Tc labeling could detect anticipated changes in liposome biodistribution up to 30 min postinjection, caused by alterations in composition or size of liposomes. Furthermore, it was capable of revealing in vivo the decrease in the stability of DMPC:DMPG liposomes when increasing amounts of GM_1 were incorporated in their bilayers. These findings, and the simplicity and the versatility of the method, suggest that labeling of preformed liposomes with ^{99m}Tc provides a tool for fast comparative screening of liposomal formulations according to their early biodistribution properties.

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