Liposomes Prepared from Synthetic Amphiphiles. II. Their Interaction with Ehrlich Ascites Tumor Cells and Tissue Distribution in Ehrlich Solid Tumor-Bearing Mice

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Received January 24, 1990

The interaction of ^{99m}Tc-labeled liposomes prepared from synthetic amphiphiles containing amino acid residues with Ehrlich ascites tumor cells *in vitro* and their tissue distributions in Ehrlich solid tumor-bearing mice were investigated. The amphiphiles used were *N,N*-didodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{12}$), *N,N*-didodecyl-N^{α}-{6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl}-L-alaninamide bromide ($CAC_2N^+C_5Ala2C_{12}$) and *S*-{1-carboxy-2-([2,3-bis(hexadecyloxy)propoxy]carbonyl)ethyl}homocysteine ($HcyM^-G2C_{16}$). Most of the radioactivity of $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ liposomes was firmly bound to Ehrlich ascites tumor cells *in vitro*. On the other hand, the accumulation of three ^{99m}Tc-labeled liposomes in the tumor of Ehrlich solid tumor-bearing mice was low (about 1% dose per gram of tissue), and most of the liposomes were taken up highly in the liver and spleen of the tumor-bearing mice. However, the radioactivity of the liposomes in the tumor, especially that of $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ liposomes, decreased more slowly with time than in the liver in up to 24 h after injection, suggesting that these liposomes were hard to separate from the tumor cells.

Keywords liposome; synthetic amphiphile; *in vitro* interaction; Ehrlich ascites tumor cell; tissue distribution; Ehrlich solid tumor-bearing mice; radiopharmaceutical

Introduction

There are many reports on the use of liposomes prepared from phosphatidylcholines as carriers of drugs and radiopharmaceuticals. However, such problems as their stability and targeting remain to be solved. Since Kunitake and Okahata[1] first reported in 1977 that artificial amphiphiles form vesicles (liposomes), new amphiphilic compounds have been synthesized and their bahavior in membrane models has been investigated.[2] Some liposomes prepared from synthetic amphiphiles might be useful as a radiopharmaceutical for the following reasons; (1) synthetic amphiphiles themselves are kept stable at room temperature, unlike phosphatidylcholines, and single-walled vesicles prepared from amphiphiles in an aqueous solution were found to be stable under ordinary conditions without additions such as cholesterol,[3] and (2) tissue distribution might be altered by changing the hydrophilic structure of an amphiphile.

We selected synthetic amphiphiles containing amino acid residues, that is, *N,N*-didodecyl-N^{α}-[6-(trimethylammonio)-hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{12}$),[3] *N,N*-didodecyl-N^{α}-{6-[dimethyl(2-carboxyethyl)ammonio]-hexanoyl}-L-alaninamide bromide ($CAC_2N^+C_5Ala2C_{12}$)[3] and *S*-{1-carboxy-2-([2,3-bis(hexadecyloxy)propoxy]-carbonyl)ethyl}homocysteine ($HcyM^-G2C_{16}$).[4] And we reported the labeling of vesicles prepared from each of the amphiphiles by using stearylamine–diethylenetriamine pentaacetic acid (SA–DTPA) as a ligand for ^{99m}Tc and their stability in saline and in serum.[5]

It is well known that phospholipid liposomes are predominantly taken up by reticuloendothelial tissues, and their behavior *in vivo* is dependent on their sizes, charges, and stability. The liposomes prepared from $N^+C_5Ala2C_{12}$, $CAC_2N^+Ala2C_{12}$ and $HcyM^-G2C_{16}$ have cationic, weak cationic and anionic charges, respectively, as described in the previous paper.[5] Both of the liposomes prepared from $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ have alanine residues, although the latter has an additional carboxyl group. On the other hand, the liposomes from $HcyM^-G2C_{16}$ have homocysteine residues. Accordingly, their physical properties are presumed to be different.

The present study was undertaken to examine the interaction of the ^{99m}Tc-labeled liposomes prepared from $N^+C_5Ala2C_{12}$, $CAC_2N^+C_5Ala2C_{12}$ and $HcyM^-G2C_{16}$ with Ehrlich ascites tumor cells *in vitro* and their tissue distribution in Ehrlich solid tumor-bearing mice, compared with the small liposomes composed of phosphatidylcholines and cholesterol. The liposomes prepared from a synthetic amphiphile are hereafter referred to 'as synthetic liposomes', and the liposomes from phosphatidylcholines and cholesterol as 'natural liposomes'.

Materials and Methods

Materials Main reagents for the synthesis of amphiphilic compounds were obtained from Kanto Chemical Co., Tokyo (di-*n*-dodecylamine and 2,3-dihydropyrane), Peptide Institute Inc., Osaka (Boc-L-alanine) and Sigma Chemical Co., St. Louis, Mo, (DL-homocysteine). Phosphatidylcholine (Phospholipid-PCE; from egg yolk) and cholesterol were the products of Nippon Fine Chemical Co., Hyogo and Sigma Chemical Co., St. Louis, Mo, respectively. 2,4-Dinitrophenol, sodium azide, cytocharasin B, and Phospholipid B-test were purchased from Wako Pure Chemical Industries, Ltd., Osaka. A ^{99}Mo–^{99m}Tc generator was purchased from Daiichi Radioisotope Lab., Tokyo. Male mice (dd/Y, 20—25 g) were obtained from Japan SLC, Inc., Hamamatsu. Other chemicals used were of guaranteed grade.

Preparation of ^{99m}Tc-Labeled Synthetic and Natural Liposomes Containing SA-DTPA $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ were synthesized by the procedure of Murakami *et al.*,[3a] $HcyM^-G2C_{16}$ by the procedure of Neumann and Ringsdorf[4] and SA–DTPA as a ligand of ^{99m}Tc by the procedure of Hnatowich *et al.*[6] The preparation of ^{99m}Tc-labeled liposomes and the determination of their sizes have been previously reported.[5]

$N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala\,2C_{12}$ were determined by the Orange II method[7] and $HcyM^-G2C_{16}$ by the ninhydrin method. Phosphatidylcholines were assayed by using Phospholipid B-test.

Labeling of SA–DTPA by ^{99m}Tc SA–DTPA (1.8 mg) was suspended in 2 ml of 0.2 M $NaHCO_3$ and sonicated to obtain a clear solution. To this solution (1 ml), 1 ml of stannous chloride solution (40 μg $SnCl_2 \cdot 2H_2O$/ml, pH 7.0) and 37 MBq (1 mCi) of $Na^{99m}TcO_4$ were added. The mixture was allowed to stand for 40 min at room temperature and filtered through a 0.2 μm membrane filter. ^{99m}Tc-Labeled SA–DTPA was purified on a Sephadex G-25 column using phosphate buffered saline. SA–DTPA was determined by the spectrophotometric method.[8]

Ehrlich Solid Tumor-Bearing Mice Mice (dd/Y, 20—25 g) bearing Ehrlich solid tumor were obtained by a subcutaneous injection of 0.2 ml of Ehrlich ascites tumor cell suspension (1×10^8 cells/ml in saline) 7 d before use.

Interaction of ^{99m}Tc-Labeled Liposomes with Ehrlich Ascites Tumor Cells Ehrlich ascites tumor cell suspension (0.5 ml) in Krebs Ringer phosphate buffer (KRP), pH 7.2, was incubated with ^{99m}Tc-labeled liposomes (0.5 ml, 6×10^5 cpm/ml, 20 μM) at 5, 20 or 37 °C for a given time up to 30 min. After adding five volumes of ice-cold KRP to the incubation mixture, it was immediately centrifuged at $650 \times g$ for 1 min. The cells were washed twice with 2 ml of ice-cold KRP and counted in a gamma counter. In the inhibitory experiment, after the preincubation of the cell suspension (0.5 ml) with 2,4-dinitrophenol (0.5 ml, final concn. 0.1 or 1 mM) at 37 °C for 10 min, the above-mentioned ^{99m}Tc-labeled liposomes (0.5 ml) were added. The mixture was incubated at 37 °C for a given time and then treated by the same procedure.

Tissue Distribution of ^{99m}Tc-Labeled Liposomes and SA–DTPA in Ehrlich Solid Tumor-Bearing Mice Tumor-bearing mice (five mice per group) were intravenously (i.v.) injected with 0.2 ml of ^{99m}Tc-labeled liposomes (5×10^7 cpm/ml in saline, 50 μg of amphiphile/ml in saline) or 0.2 ml of ^{99m}Tc-labeled SA–DTPA (3×10^7 cpm/ml in saline, 100 μg/ml in saline). After collecting blood from the carotid artery under etherization at a given time after injection, solid tumor and other organs were excised and weighed. The radioactivity was counted in a gamma counter (Beckman Gamma 5500).

Distribution of ^{99m}Tc-Labeled Liposomes in Blood Cells and Plasma of Mice After the intravenous injection of ^{99m}Tc-labeled liposomes, blood was collected from an inferior vein with a heparin-treated syringe under etherization at a given time, and immediately centrifuged at $800 \times g$ for 10 min. The blood cells were washed once with saline. The supernatant was combined with the plasma fraction. The radioactivity in the blood cells and the plasma was counted in the gamma counter described above.

Results and Discussion

***In Vitro* Interaction of ^{99m}Tc-Labeled Liposomes with Ehrlich Ascites Tumor Cells** There are several mechanisms of interaction between liposomes and cells; stable adsorption, endocytosis, fusion and lipid exchange.[9] In this study, the interaction of ^{99m}Tc-labeled synthetic liposomes with Ehrlich ascites tumor cells *in vitro* was investigated. Figure 1 shows the time-courses of the uptake of ^{99m}Tc-labeled liposomes in the tumor cells at 5, 20 and 37 °C, including that of ^{99m}Tc-labeled SA–DTPA alone (Fig. 1A). Among these liposomes, the radioactivity of $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ liposomes was high in Ehrlich tumor cells, but that of $HcyM^-G2C_{16}$ and natural liposomes, and SA–DTPA itself was low. The uptake was hardly affected by the incubation temperature. The high uptake of $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ liposomes might depend on their electric charge or on the structure of the hydrophilic region.

The effect of 2,4-dinitrophenol (DNP), which is known as an uncoupler of oxidative phosphorylation, was examined in order to study the participation of a metabolic energy in the interaction of liposomes with tumor cells (Fig. 2). The interaction was made insensitive to the energy inhibitor by incubating the cells for 10 min with 0.1 or 1.0 mM DNP before the addition of the liposomes. No effect of sodium azide or cytocharasin B was observed (data not shown).

Since DNP and sodium azide are typical energy inhibitors of cellular metabolism, these results strongly suggested that the interaction is not energy dependent, as reported on the interaction of liposomes with Walker tumor cells.[10] Therefore, an active process like endocytosis should be ruled out. This is not incompatible with the lack of the effect of cytocharasin B on the interaction of liposomes because it is known as the inhibitor of endocytosis.[11] This might also be supported by the observation that the interaction of liposomes with the tumor cells was independent from temperature, as mentioned above. The radioactivity in/on Ehrlich tumor cells scarcely decreased by washing the cells. These results indicated either the strong adsorption of

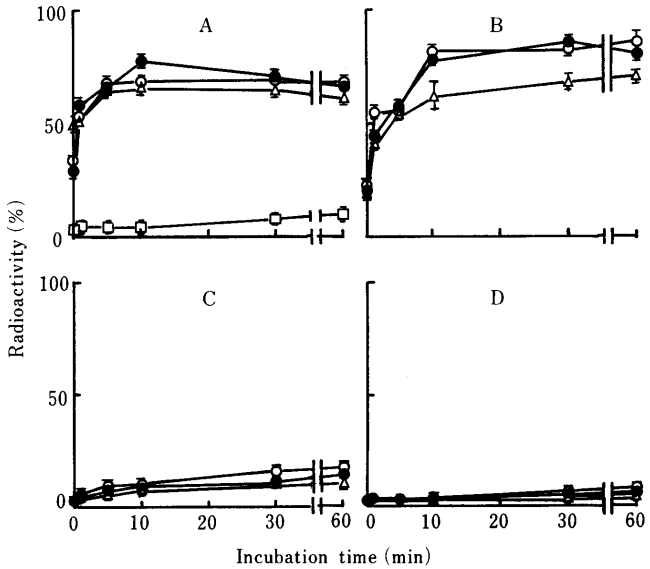

Fig. 1. Interaction of ^{99m}Tc-Labeled Liposomes with Ehrlich Ascites Tumor Cells

^{99m}Tc-Labeled liposomes (0.5 ml, 20 μM, 6×10^5 cpm/ml) were incubated with 0.5 ml of Ehrlich ascites tumor cells (10^8 cells/ml) at 5, 20 or 37° C for a given time and then treated as described in the text. A, $N^+C_5Ala2C_{12}$ liposomes; B, $CAC_2N^+C_5Ala2C_{12}$ liposomes; C, $HcyM^-G2C_{16}$ liposomes; D, natural liposomes. ○, 37 °C; ●, 20 °C; △, 5 °C; □, SA–DTPA (0.5 ml, 1.5×10^5 cpm/ml, 60 μM) at 37 °C.

$$\text{Radioactivity} = \frac{\text{radioactivity in tumor cells}}{\text{radioactivity in a tube}} \times 100\ (\%).$$

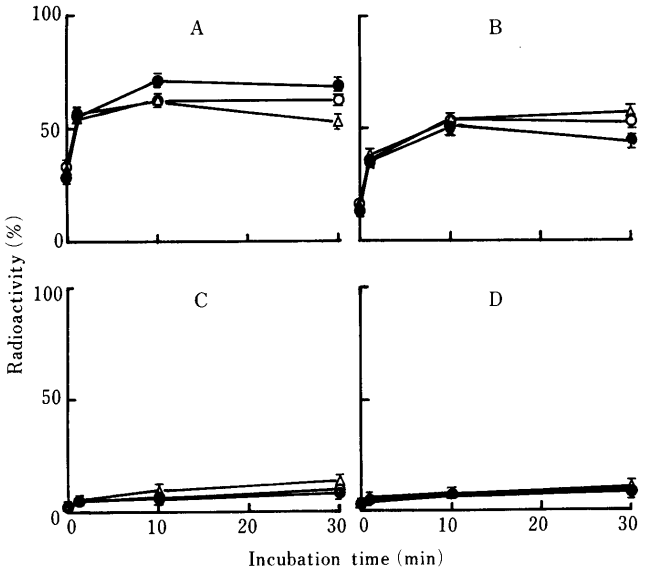

Fig. 2. Effect of 2,4-Dinitrophenol on Interaction of ^{99m}Tc-Labeled Liposomes with Ehrlich Ascites Tumor Cells

Ehrlich cells (0.5 ml, 10^8 cells/ml) preincubated with 0.5 ml of 0.2 or 2.0 mM 2,4-dinitrophenol (DNP) at 37 °C for 10 min and 0.5 ml of ^{99m}Tc-labeled liposomes (0.5 ml, 30 μM, 6×10^5 cpm/ml) were incubated at 37 °C and then treated as described in the text. A, $N^+C_5Ala2C_{12}$ liposomes; B, $CAC_2N^+C_5Ala2C_{12}$ liposomes; C, $HcyM^-G2C_{16}$ liposomes; D, natural liposomes. △, control; ○, 0.1 mM DNP; ●, 1.0 mM DNP.

$$\text{Radioactivity} = \frac{\text{radioactivity in tumor cells}}{\text{radioactivity in a tube}} \times 100\ (\%).$$

TABLE I. Tissue Distribution of ^{99m}Tc-Labeled Synthetic and Natural Liposomes in Tumor-Bearing Mice

	% dose/g of tissue			
	Time after i.v. injection (h)			
	1	3	6	24
$N^+C_5Ala2C_{12}$ liposomes				
Liver	27.0 ±3.6	28.3 ±1.0	18.0 ±3.8	11.9 ±1.4
Spleen	9.00±0.78	9.12±0.36	6.58±2.75	4.39±0.33
Lung	1.47±0.28	1.36±0.23	0.58±0.20	0.66±0.26
Kidney	3.21±0.74	3.63±0.41	3.11±0.90	2.60±0.89
Muscle	0.17±0.07	0.14±0.04	0.12±0.03	0.09±0.05
Stomach	0.32±0.11	0.38±0.08	0.39±0.12	0.85±0.37
Brain	0.08±0.02	0.05±0.01	0.03±0.01	0.01±0.01
Pancreas	0.30±0.09	0.21±0.04	0.19±0.09	0.10±0.02
Blood	2.63±0.19	1.23±0.23	0.58±0.18	1.41±0.45
Tumor	0.97±0.18	1.32±0.24	0.85±0.24	0.89±0.05
$CAC_2N^+C_5Ala2C_{12}$ liposomes				
Liver	33.5 ±2.8	26.2 ±1.3	20.3 ±1.8	10.4 ±1.2
Spleen	6.25±2.47	8.45±3.45	6.86±3.21	5.39±1.27
Lung	2.49±0.66	1.44±0.26	1.42±0.42	0.94±0.06
Kidney	2.51±0.13	3.31±0.59	3.71±0.04	3.05±0.04
Muscle	0.16±0.03	0.17±0.08	0.10±0.02	0.03±0.02
Stomach	0.41±0.07	0.46±0.12	0.67±0.32	1.18±0.10
Brain	0.04±0.00	0.03±0.01	0.03±0.01	0.02±0.01
Pancreas	0.22±0.02	0.24±0.06	0.19±0.02	0.10±0.02
Blood	1.01±0.08	0.65±0.03	0.48±0.10	0.44±0.14
Tumor	0.51±0.20	0.65±0.20	0.90±0.29	0.82±0.27
$HcyM^-G2C_{16}$ liposomes				
Liver	30.7 ±2.4	26.6 ±3.5	25.8 ±2.9	14.6 ±1.7
Spleen	7.08±2.64	7.54±1.59	5.72±1.42	4.29±0.85
Lung	1.34±0.19	0.96±0.16	0.68±0.13	0.27±0.06
Kidney	3.44±0.35	3.78±0.35	3.92±0.52	2.10±0.27
Muscle	0.25±0.07	0.32±0.26	0.10±0.02	0.05±0.01
Stomach	7.48±0.01	7.63±1.22	5.30±0.58	1.45±0.19
Brain	0.07±0.02	0.05±0.01	0.03±0.01	0.01±0.00
Pancreas	0.40±0.05	0.30±0.04	0.20±0.03	0.09±0.02
Blood	1.11±0.14	0.78±0.07	0.56±0.08	0.19±0.02
Tumor	0.86±0.17	0.67±0.11	0.66±0.07	0.52±0.14
Natural liposomes				
Liver	22.8 ±4.1	16.5 ±0.5	21.5 ±1.8	8.05±0.60
Spleen	16.8 ±3.7	8.74±2.55	9.93±1.97	7.33±1.73
Lung	2.35±0.28	1.21±0.02	1.45±0.37	0.87±0.03
Kidney	7.27±0.30	5.94±1.23	5.55±0.28	2.97±0.48
Muscle	0.48±0.01	0.34±0.06	0.88±0.11	0.97±0.16
Stomach	1.59±0.21	1.39±0.25	2.45±0.70	1.28±0.39
Brain	0.14±0.01	0.14±0.01	0.26±0.04	0.25±0.03
Pancreas	0.80±0.17	0.43±0.02	0.82±0.11	0.76±0.33
Blood	5.34±1.47	2.82±0.75	1.13±0.14	0.59±0.32
Tumor	3.26±0.51	2.38±0.17	2.84±0.20	1.35±0.06

Each value represents the mean±S.D. for five mice and is normalized to body weight of 25 g.

$N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ liposomes to the cell surface, liposome-cell fusion, or lipid exchange.

Tissue Distribution of ^{99m}Tc-Labeled Liposomes in Ehrlich Solid Tumor-Bearing Mice Since $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ liposomes showed high interaction with Ehrlich tumor cells *in vitro* as mentioned above, the tissue distribution of the synthetic liposomes in Ehrlich solid tumor-bearing mice was compared with that of the natural liposomes which were composed of phosphatidylcholines and cholesterol (1 : 1 molar ratio) (Table I). The results are expressed as a percentage of the dose per gram of tissue at a given time up to 24 h. The mean diameters of these synthetic liposomes used in this study fell in the range of 50 to 100 nm[5] and they were considered to belong to small unilamellar vesicles (SUVs) (30—60 nm). The size of natural liposomes used as reference was 50—100 nm in diameter.[5] Synthetic liposomes were taken up highly in liver and spleen belonging to reticuloendothelial tissues. When the percentages of the injected dose per organ were measured, 69—80% of the injected radioactivity in $N^+C_5Ala2C_{12}$, $CAC_2N^+C_5Ala2C_{12}$ and $HcyM^-G2C_{16}$ liposomes were observed in the liver and spleen of mice 1 h after injection (Table II). There was a continuous decline of these values with time.

TABLE II. Distribution of ^{99m}Tc-Labeled Synthetic and Natural Liposomes in Whole Liver and Spleen of Ehrlich Solid Tumor-Bearing Mice

	% dose/organ			
	Time after i.v. injection (h)			
	1	3	6	24
$N^+C_5Ala2C_{12}$ liposomes				
Liver	67.0 ±8.0	64.4 ±4.8	39.4 ±7.8	29.2 ±3.3
Spleen	2.37±0.32	2.05±0.42	1.47±0.85	1.33±0.14
$CAC_2N^+C_5Ala2C_{12}$ liposomes				
Liver	75.5 ±5.5	67.8 ±4.2	52.4 ±6.0	26.8 ±2.6
Spleen	2.76±0.95	2.69±1.26	2.12±0.96	1.66±0.46
$HcyM^-G2C_{16}$ liposomes				
Liver	77.9 ±6.1	65.0 ±11.1	64.0 ±7.8	36.4 ±5.0
Spleen	1.89±0.53	1.89±0.41	1.80±0.43	1.30±0.34
Natural liposomes				
Liver	54.4 ±11.1	44.7 ±1.8	53.0 ±6.0	19.0 ±2.0
Spleen	4.99±1.33	2.76±0.48	3.69±1.65	2.23±0.83

Each value represents the mean±S.D. for five mice and is normalized to body weight of 25 g.

TABLE III. Tissue Distribution of ^{99m}Tc-Labeled SA–DTPA in Tumor-Bearing Mice

	% dose/g of tissue			
	Time after i.v. injection (h)			
	1	3	6	24
Liver	3.88±0.3	3.17±0.3	3.17±0.3	1.99±0.3
Spleen	0.91±0.06	0.65±0.17	0.54±0.05	0.36±0.05
Lung	1.65±0.36	0.87±0.16	0.59±0.08	0.22±0.04
Kidney	6.40±0.48	4.39±0.85	3.96±0.13	2.63±0.45
Muscle	0.39±0.06	0.21±0.07	0.19±0.03	0.03±0.01
Stomach	7.77±2.79	5.81±0.97	4.66±1.03	1.55±0.83
Brain	0.08±0.01	0.06±0.01	0.04±0.01	0.03±0.01
Pancreas	0.42±0.04	0.26±0.01	0.23±0.04	0.04±0.02
Blood	2.58±0.16	1.32±0.12	0.86±0.10	0.40±0.05
Tumor	1.54±0.26	1.10±0.12	1.09±0.24	0.79±0.22

Each value represents the mean±S.D. for five mice and is normalized to body weight of 25 g.

In general, the accumulation of the radioactive marker encapsulated in liposomes can be explained in two ways,[12] that is, one characterized by the distribution of intact liposomes and the other by the distribution of a released marker. When the liposomes are leaky in the blood, the tissue distribution data exhibit a composite distribution of the intact liposomes and the released marker. Therefore, it is necessary to examine the tissue distribution of ^{99m}Tc-SA–DTPA as a released marker *in vivo*. As shown in Table III, the accumulation of ^{99m}Tc-SA–DTPA was low in the liver and spleen, but high in the stomach. Therefore, it was presumed that the high accumulation of ^{99m}Tc-labeled liposomes in the liver and spleen reflected mainly the distribution of intact liposomes.

It was reported that liposome size, charge and stability have some effects on the retention time of liposomes in the blood clearance.[13] Gregoriadis *et al.*[13c] have suggested that the broader tissue distribution of small liposomes could be attributable to the slower rate of elimination of small liposomes from the blood, which would undergo transcapillary passage, escape from the liver and spleen, and reach alternative tissues. Hwang *et al.*[12] and Ogihara *et al.*[14] showed that the uptake of SUVs encapsulating ^{67}Ga-nitrilotriacetic acid in the liver of normal mice was lower than that of the multilamellar vesicles (MLVs). In the present study, ^{99m}Tc-labeled synthetic and natural liposomes were predominantly taken up in the liver. Similar phenomena were observed with MLVs,[12,14] although the sizes of ^{99m}Tc-labeled synthetic and natural liposomes were in the range of those of SUVs. Therefore, our results can hardly be interpreted on the basis of the liposome size. It was presumed that the difference in their liver uptakes was due to the localization of the radioactive marker in the liposomes. Hwang *et al.*[12] and Ogihara *et al.*[14] examined the tissue distribution of SUVs *in vivo* by using markers which are water-soluble and encapsulated in the aqueous space. This marker is likely to be released even when the liposomes are slightly broken and excreted rapidly from liver cells, showing a low uptake in the liver. On the other hand, the marker used in this experiment, ^{99m}Tc-labeled SA–DTPA, might be bound with the lipid layer by its hydrophobic interaction.[6] Therefore, even if the liposomes were broken into fragments in the liver cells, it was assumed that the radioactive marker was hardly released from the fragments. In other words, since the radioactive marker might behave together with the fragments which were about the same size as that of the original liposomes, the *in vivo* behavior of the radioactive marker embedded in the fragments is apparently similar to that in the intact liposomes. This is considered to be one of the explanations for the high accumulation in the liver and spleen.

The tumor accumulation of three ^{99m}Tc-labeled synthetic liposomes was about 1% dose per gram tissue and lower than that of ^{99m}Tc-labeled natural liposomes (Table I). The radioactivity of ^{99m}Tc-labeled $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ liposomes in the tumor hardly changed and that of $HcyM^-G2C_{16}$ liposomes decreased slightly up to 24 h after the injection, compared with the decrease in the liver with time. This suggested that the synthetic liposomes were hard to separate from the tumor cells after their binding. The behavior of $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ liposomes corresponded to the strong interaction with tumor cells *in vitro*, as shown in Figure 1, but that of $HcyM^-G2C_{16}$ liposomes could not be interpreted.

Liposomes labeled with γ-emitter nuclide, such as ^{99m}Tc, ^{67}Ga or ^{111}In, have been studied as a tumor-detecting agent in nuclear medicine.[14,15] It was reported that the uptake of SUVs in the tumor of animals was high[14,15a,c] and much higher than that of MLVs.[14] This uptake was speculated as follows: SUVs having their slow clearance from blood can pass out of the vascular bed and be taken up by surrounding tissues,[16] especially tumor, because of the enhanced capillary permeability of the blood vessels of tumor in comparison with normal tissue.[17] Our results showed a low tumor uptake (about 1% of dose per gram of tissue) in spite of small liposomes, in contrast to the report that the tumor uptake of SUVs was 10%—13% of dose per gram of tissue.[14] Most radioactivity of the ^{99m}Tc-labeled synthetic liposomes embedded SA–DTPA was accumulated in liver and spleen within a short time, resulting in a low concentration in the blood. This is one main reason why the liposomes were not taken up in the tumor. However, the difference between our result and Ogihara's result[14] in the tumor uptake could still not be elucidated.

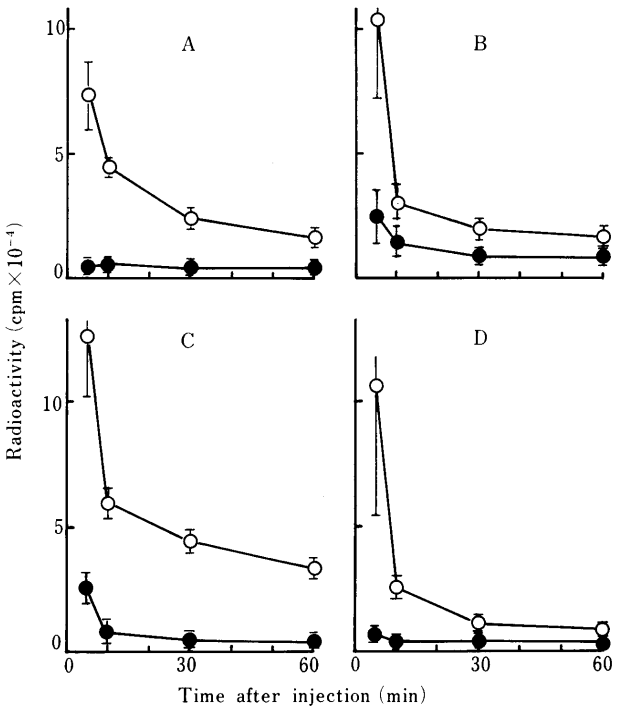

Fig. 3. Distribution of ^{99m}Tc-Labeled Liposomes in Mouse Blood after Injection

^{99m}Tc-Labeled liposomes (0.2 ml, 75 μM, 1.5×10^7 cpm/ml) were intravenously injected into mice. Blood was collected from inferior *vena cava* in heparinized syringe and centrifuged at $800\times g$ for 10 min at 4 °C. A, $N^+C_5Ala2C_{12}$ liposomes; B, $CAC_2N^+C_5Ala2C_{12}$ liposomes; C, $HcyM^-G2C_{16}$ liposomes; D, natural liposomes. ○, plasma; ●, blood cells.

There are many reports on the effects of the surface charge of liposomes on their tumor uptake.[14,15] It was reported that the tumor uptake was best with small negatively charged liposomes,[15a] positive liposomes [15b] and neutral liposomes.[15c] On the other hand, it was reported that there is no significant difference in tumor uptake between liposomes with different surface charges in Ehrlich solid tumor-bearing mice.[14] Our results showed that the respective charges of ^{99m}Tc-labeled synthetic liposomes had no significant effects on the tumor uptake. The reports described above and our results could not clarify the effect of a surface charge of liposomes on the tumor uptake.

The rate of blood clearance of the synthetic liposomes was fast as shown in Table I. Therefore, the behaviors of synthetic liposomes and natural liposomes in blood were examined within 60 min after injection (Fig. 3). A large portion of the radioactivity of ^{99m}Tc-labeled liposomes 5 min after injection was observed in the fraction of plasma, not of blood cells. The degree of the interactions of respective liposomes, especially $N^+C_5Ala2C_{12}$ and natural liposomes, with blood cells seemed to be low.

In conclusion, the tested synthetic liposomes themselves cannot be applied to radiopharmaceuticals used for diagnosis of other tissues except liver, because they were highly taken up in reticuloendothelial tissues, especially the liver. However, $N^+C_5Ala2C_{12}$ liposomes which were stable in an aqueous solution[5] were firmly bound to tumor cells *in vitro*, had a low interaction with blood cells and were hard to separate from tumor cells *in vivo*. Therefore, it is expected that $N^+C_5Ala2C_{12}$ liposomes will be accumulated in other tissue such as tumor tissue by the addition of some targeting groups to the liposomes for the purpose of decreasing the reticuloendothelial system uptake.

Acknowledgment The authors are grateful to Miss M. Kobori, Miss Y. Nakamura and Mr. N. Kawaguchi of this laboratory for their technical assistance.

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