
Increased Delivery of Gallium-67 to Tumors Using Serum-Stable Liposomes

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Gallium-67 chelated to nitrilotriacetic acid was encapsulated in liposomes composed of various phospholipids, and ^{67}Ga delivery potential to the tumor after intravenous injection of these liposomes was examined. Tumor uptake of the liposomes themselves and their stability in the serum were also studied. It was found that liposomes composed of distearoylphosphatidylcholine, diarachidoylphosphatidylcholine, or sphingomyelin with cholesterol (molar ratio of phospholipid:cholesterol, 2:1) could be taken by the tumor effectively and could deliver large amounts of ^{67}Ga to the tumor. They could also give high ^{67}Ga accumulation ratios (tumor to the other tissues). The study of liposomal stability in the serum suggested that the marked ^{67}Ga accumulation in the tumor resulted from the serum stability of the liposomal bilayer, i.e., the stable liposomes in the blood circulation could reach the tumor in large quantities after i.v. injection. These observations indicate that liposomes with an appropriate lipid composition may be an excellent tool to accumulate ^{67}Ga in tumors.

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Liposomes, or artificial lipid vesicles, are one of the most promising carriers for drugs, enzymes, and other biologically important materials. Both water-soluble and lipid-soluble materials can be encapsulated into liposomes, either in the aqueous or lipid phase. For many medical applications liposomes can be viewed as pharmacologic capsules (1) and, in the field of nuclear medicine, attempts have been made to apply liposomes as radiopharmaceuticals by encapsulating gamma-emitters, positron-emitters, or nuclear magnetic resonance markers. Various attempts to apply liposomes in the diagnosis of lymph system disorders, myocardial infarcts, reticuloendothelial system disorders, inflammations, tumor, etc., have been reported (2-5). Recently, several clinical trials have also been reported (6-8), though work is still at an early stage.

We have been investigating the application of liposomes in specific tumor imaging, by encapsulating gallium-67 (^{67}Ga) chelated to nitrilotriacetic acid, and have found that certain liposomes, for instance, small unilamellar vesicles prepared from distearoylphosphatidylcholine and cholesterol, could deliver large amounts of ^{67}Ga to the tumor (9,10). In order to apply liposomes

in tumor imaging, we have to use liposomes that can deliver large amounts of ^{67}Ga to the tumor specifically. Initially, we tried to enhance the ^{67}Ga delivery potential to the tumor by varying the chemical composition of the liposomes.

Liposomes can be characterized in terms of chemical composition, size, number of membrane bilayers, and surface charges. Biodistributions of liposomes after i.v. injection are strongly influenced by such factors. In particular, chemical composition largely determines liposome stability, which greatly influences the delivery of the liposome contents to various tissues (11-13). We expected that such factors would also influence the tumor uptake and ^{67}Ga delivery potential of liposomes. In this work we examined the ^{67}Ga delivery potential to tumors by using liposomes composed of various phospholipids. The relations between ^{67}Ga delivery potential, tumor uptake of the liposomes themselves, and the stability of the liposomal bilayer in the serum are discussed.

MATERIALS AND METHODS

Preparation of Liposomes

Phospholipids used in this work were as follows (Sigma Chemical Corp., St. Louis, MO): L- α -phosphatidylcholine from frozen egg yolk (PC), L- α -dimyristoylphosphatidylcholine (DMPC), L- α -dipalmitoylphosphatidylcholine (DPPC),

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L- α -distearoylphosphatidylcholine (DSPC), *L*- α -diarachidoylphosphatidylcholine (DAPC), and sphingomyelin from bovine brain (SM). Liposomes were prepared as previously described (10) with slight modifications. Chloroform solutions of phospholipids and cholesterol (CH) (molar ratio 2:1) were evaporated to dryness under a stream of nitrogen. When the lipid phase of liposomes was traced, cholesteryl [1- 14 C]oleate (58 mCi/mmol) was included in the mixture. The evaporation was performed at 37°C when PC and DMPC were used, at 45°C when DPPC and SM were used, and at 60°C when DSPC and DAPC were used. After storing the dried lipid films at reduced pressure overnight, 150 mM NaCl/5 mM sodium phosphate (pH 7.4) (PBS) containing 1 mM NTA was added, then the solutions were warmed at the above temperatures, suspended by vortexing and sonicated. Sonication was carried out at 20 kHz, a power level of 45 W for 60 min (continuous) with a titanium microtip at the above temperatures. Then, it was centrifuged at 100,000 *g* for 1 hr. Liposomes contained in the supernatant were almost all small unilamellar vesicles (14), and their mean diameter was estimated to be ~60 nm by electron microscopy of preparations negatively stained with potassium phosphotungstate (9). Untrapped materials were removed by passage of the liposomes through a Sephadex G-50 column (1 \times 30 cm) in PBS. Liposomes were eluted immediately after the void volume. When 67 Ga was encapsulated, the loading method using 67 GaCl₃ (Nihon Medipysics Co., Ltd., Takarazuka, Japan) described earlier was employed (10). Judging from phosphorous content determinations, incorporation of phospholipid in the final preparations was 69.5–73.4% of the amount used when PC and DMPC were used, 52.2–56.3% when DPPC, and 23.2–29.8% when DSPC, DAPC, and SM were used.

Transplantation of Ehrlich Solid Tumor

Ehrlich solid tumor was used as a tumor model. Male ddY mice, weighing 28–30 g, were subcutaneously injected in the left hind leg with 4×10^6 cells. At 9 to 10 days after inoculation, the tumor weighed between 0.1 and 0.5 g and rarely included necrotic areas.

Tissue Distribution of 67 Ga and 14 C Radioactivity After Intravenous Injection of Liposomes

Liposomes encapsulating [67 Ga]NTA (0.3 μ mol as phospholipid and 0.5–1.0 μ Ci/0.2 ml/mouse) were injected into the tail vein of the tumor-bearing mice. Gallium-67 citrate (Nihon Medipysics Co., Ltd., Takarazuka, Japan) and [67 Ga]NTA (1 μ Ci/0.2 ml/mouse) were administered in a similar manner. Tissue distribution of 67 Ga radioactivity was evaluated as described earlier (9). Data were expressed as tissue distribution rate (% administered dose/g tissue), as tumor-to-tissue concentration ratios, and as tumor index (TI). The latter criterion has been proposed for the evaluation of the practical utility of a radiotracer for imaging, and in the product of the tumor-to-blood ratio (T/B) and the tumor uptake (% administered dose/g tumor) ($TI = T/B \times \% \text{ dose/g}$) (15). When liposomes labeled with cholesteryl [1- 14 C]oleate were injected (0.3 μ mol as phospholipid and 0.15 μ Ci/0.2 ml/mouse), whole tissues or aliquots (~0.2 g) were minced in the counting vials. Protosol (NEN Research Products, Boston, MA) (1.5 ml per vial) was added and each sample was incubated at 55°C overnight or until a clear solution was obtained. Blood samples were treated as follows: 0.1 ml of blood was added to 0.5 ml

of Protosol:ethanol (1:2) and warmed at 55°C for 30 min. Then 0.2 ml of 30% hydrogen peroxide was added and each mixture was incubated for more 30 min. After cooling, 8 ml of Biofluor (NEN Research Products, Boston, MA) was added and shaken vigorously. The samples were neutralized with 2 N NaOH and carbon-14 (14 C) radioactivity was counted in a liquid scintillation counter. The recovery of 14 C radioactivity by this method ranged 97% to 101% with an average of 99.2%.

Stability of Liposomes in Serum in Vitro

Liposomes encapsulating [67 Ga]NTA were incubated at 37°C with fresh mouse serum. The volume ratio of serum/liposomes was 5, i.e., similar to that expected upon i.v. injection of mice with 0.2 ml of liposomes (assuming 2 ml blood volume and 50% hematocrit) (14). After various time intervals, aliquots were taken and passed through a Sepharose CL-4B column (0.8 \times 25 cm) in PBS. Fractions of 0.5 ml each were collected and the 67 Ga radioactivity in each fraction was counted in a gamma counter.

RESULTS

Figure 1 shows the uptake and retention of 67 Ga radioactivity in the tumor and blood of mice bearing Ehrlich solid tumor after the i.v. injection of liposomes prepared from various phospholipids with CH (molar ratio 2:1) and encapsulating [67 Ga]NTA. It was found that liposomal lipid composition had a great influence upon 67 Ga uptake in the tumor and blood. When the liposomes were composed of PC or DMPC with CH (PC-liposomes or DMPC-liposomes, respectively) blood clearance of 67 Ga was rapid, while the tumor uptake was low and decreased with time. In contrast, blood retention of DSPC-, DAPC-, and SM-liposomes was longer and tumor uptake of 67 Ga increased, reached a peak and then slowly decreased. Tumor uptake peaked at 12.6% of the administered dose per gram (% AD/g) at 12 hr after injection of DSPC-liposomes, while it peaked at 13.3% at 12 hr with DAPC-liposomes and at 17.1% at 24 hr with SM-liposomes. These values are three to four times higher than those with PC-, DMPC-, and DPPC-liposomes.

Table 1 shows the sequential changes of the tumor-to-blood ratio (T/B) and tumor index (TI) of 67 Ga radioactivity after the administration of the liposomes having the same composition as those in Figure 1. When 67 Ga was encapsulated in PC-, DMPC-, and DPPC-liposomes, T/B increased gradually, and maximal T/B obtained at final period (72 hr) were ~6 to 7. When 67 Ga was encapsulated in DSPC-, DAPC-, and SM-liposomes, T/B increased more rapidly. The values have already reached 6 to 7 at 24 hr after the administration, and maximal T/B were 10 to 14 (at 48 hr or 72 hr). With every liposomes, the changes of TI with time were similar to those of T/B, respectively. However, TI of DSPC-, DAPC-, and SM-liposomes increased much steeper than their T/B. TI of these liposomes were much higher than that of the other at and after 24 hr.

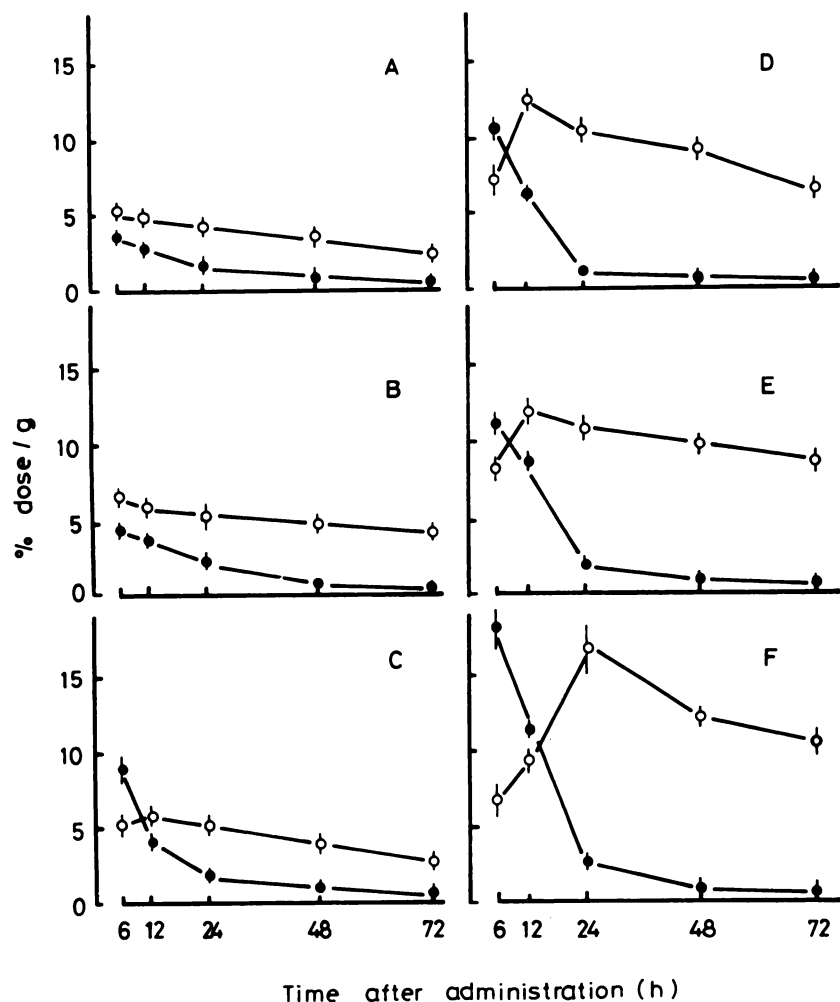


FIGURE 1
Uptake and retention of ^{67}Ga radioactivity in tumor and blood after the administration of liposomes composed of various phospholipids with cholesterol and encapsulating ^{67}Ga NTA. Liposomes composed of PC (A), DMPC (B), DPPC (C), DSPC (D), DAPC (E), and SM (F) with CH (phospholipid:CH molar ratio 2:1) were administered i.v. to mice bearing Ehrlich solid tumor. (O) Tumor; (●) Blood.

Table 2 shows the tissue distributions and the tumor-to-tissue ratios of ^{67}Ga radioactivity 24 hr after i.v. injection of liposomes prepared from various phospholipids with CH and encapsulating ^{67}Ga NTA (as in Figure 1 and Table 1). It was found that ^{67}Ga radioactivity concentrated mainly in the liver and spleen, in addition to the tumor, with all of the liposomes. Gallium-67 uptake in some tissues (e.g., spleen or muscle) was influenced by liposomal lipid composition, as that in the tumor and blood was. However, the effects on them were much less striking compared with those on the tumor and blood. DSPC-, DAPC-, and SM-liposomes gave much higher tumor-to-tissue ratios than the other liposomes at this time point. When SM-liposomes were used, the ratios to all tissues were over 1.00.

The tissue distributions and the tumor-to-tissue ratios of ^{67}Ga radioactivity 24 hr after i.v. injection of free (not encapsulated) ^{67}Ga NTA and ^{67}Ga citrate are shown in Table 3. The tissue distributions of these agents were similar, with both exhibiting a distribution different from that of any liposomes at the same point. Tumor uptake was $\sim 4\%$ AD/g and it was nearly the maximal value of what these agents could give. T/B and TI after the administration of ^{67}Ga NTA and

^{67}Ga citrate also increased with time, but the values were similar or a little lower than those of PC-, DMPC-, and DPPC-liposomes at every time point tested (data not shown).

Table 4 shows the tissue distributions of ^{14}C radioactivity 24 hr after i.v. injection of various liposomes with cholesteryl $[1-^{14}\text{C}]$ oleate in their lipid phases. Carbon-14 radioactivity uptake in the tumor was higher than that in the other tissues, with all of the liposomes. The tissue distribution of ^{14}C radioactivity did not necessarily agree with that of ^{67}Ga (Table 2). However, ^{14}C tumor uptake of DSPC-, DAPC-, and SM-liposomes was higher than that of the other three liposomes, likewise as ^{67}Ga tumor uptake of the former was higher than that of the latter.

Liposome stability in vitro was studied by chromatographic separation of liposome-encapsulated ^{67}Ga and leaked ^{67}Ga on Sepharose CL-4B after the incubation of liposomes and serum at 37°C . The chromatogram is shown in Figure 2. Similar profiles were observed with liposomes of all compositions tested. The ^{14}C radioactivity, due to cholesteryl $[1-^{14}\text{C}]$ oleate added as a marker of the liposomal lipid phase, indicates that liposomes were eluted immediately after the void vol-

TABLE 1
Tumor-to-Blood Ratio and Tumor Index of ^{67}Ga Radio-Activity After the Administration of Liposomes Composed of Various Phospholipids and Cholesterol (Molar Ratio 2:1) and Encapsulating [^{67}Ga]NTA

Phospholipid	Tumor-to-blood ratio (Tumor index)				
	Time after administration				
	6 hr	12 hr	24 hr	48 hr	72 hr
PC	1.33 (6.71)	1.51 (7.29)	2.39 (10.16)	4.26 (15.26)	6.83 (18.33)
DMPC	1.23 (7.81)	1.34 (7.37)	3.18 (16.17)	5.00 (25.00)	7.06 (33.88)
DPPC	0.62 (3.33)	1.36 (7.83)	3.38 (17.37)	4.92 (19.15)	5.88 (22.88)
DSPC	0.69 (5.14)	2.09 (26.29)	7.01 (76.13)	13.50 (123.93)	12.00 (86.40)
DAPC	0.74 (6.12)	1.51 (19.57)	6.20 (76.45)	10.09 (101.81)	10.38 (88.32)
SM	0.35 (2.28)	0.67 (5.37)	5.71 (97.86)	13.29 (164.27)	13.76 (142.00)

ume, in fractions 7 to 12. Gallium-67 radioactivity showed two peaks, at fractions 7 to 12 and 16 to 24. The first peak represents liposome-encapsulated ^{67}Ga and the second, leaked ^{67}Ga . Serum proteins were eluted in fractions 13 to 19. In the following experiments, liposome stability was evaluated in terms of ^{67}Ga reten-

TABLE 3
Tissue Distribution of ^{67}Ga Radioactivity at 24 hr After the Administration of Free [^{67}Ga]NTA and [^{67}Ga]Citrate

Tissue	[^{67}Ga]NTA		[^{67}Ga]citrate	
	% dose/g	Tumor/tissue ratio	% dose/g	Tumor/tissue ratio
Tumor	4.09 \pm 0.43	1.00	4.30 \pm 0.38	1.00
Liver	4.02 \pm 0.44	1.01	5.62 \pm 0.58	0.76
Spleen	2.68 \pm 0.38	1.53	3.64 \pm 0.23	1.18
Kidney	3.99 \pm 0.50	1.03	5.21 \pm 0.35	0.83
Lung	2.63 \pm 0.29	1.56	2.55 \pm 0.24	1.68
Heart	0.96 \pm 0.12	4.26	1.26 \pm 0.16	3.41
Muscle	0.43 \pm 0.09	9.51	0.55 \pm 0.09	7.82
Blood	1.93 \pm 0.14	2.12	2.26 \pm 0.33	1.90

Each value represents the mean \pm s.d. for seven animals.

tion in liposomes, that is, the ratio of ^{67}Ga radioactivity in the liposomal fraction to the total activity.

Figure 3 shows the stability of various liposomes having the same compositions as those in Figure 1. When PC-, DMPC-, or DPPC-liposomes were used, leakage of ^{67}Ga from the liposomes was observed in both PBS and serum. PC- and DMPC-liposomes scarcely retained ^{67}Ga when incubated with serum. In contrast, DSPC-, DAPC-, and SM-liposomes were fairly stable during incubation with PBS or serum. DAPC-

TABLE 2
Tissue Distribution of ^{67}Ga Radioactivity at 24 hr After the Administration of Liposomes Composed of Various Phospholipids and Cholesterol (molar ratio 2:1) and Encapsulating [^{67}Ga]NTA^a

Tissue	PC-liposomes		DMPC-liposomes		DPPC-liposomes	
	% dose/g	Tumor/tissue ratio	% dose/g	Tumor/tissue ratio	% dose/g	Tumor/tissue ratio
Tumor	4.25 \pm 0.63	1.00	5.25 \pm 0.89	1.00	5.14 \pm 2.26	1.00
Liver	17.04 \pm 1.77	0.25	12.27 \pm 2.21	0.43	15.00 \pm 1.25	0.34
Spleen	27.92 \pm 8.19	0.15	20.14 \pm 1.81	0.26	22.34 \pm 3.29	0.23
Kidney	4.42 \pm 0.60	0.96	4.68 \pm 0.65	1.12	4.35 \pm 0.89	1.18
Lung	2.01 \pm 0.26	2.11	2.24 \pm 0.20	2.34	1.77 \pm 0.12	2.90
Heart	0.77 \pm 0.01	5.52	0.97 \pm 0.15	5.41	1.02 \pm 0.21	5.04
Muscle	0.29 \pm 0.03	14.66	0.40 \pm 0.07	13.13	0.31 \pm 0.02	16.58
Blood	1.78 \pm 0.17	2.39	1.65 \pm 0.30	3.18	1.52 \pm 0.27	3.38

Tissue	DSPC-liposomes		DAPC-liposomes		SM-liposomes	
	% dose/g	Tumor/tissue ratio	% dose/g	Tumor/tissue ratio	% dose/g	Tumor/tissue ratio
Tumor	10.86 \pm 1.20	1.00	12.33 \pm 1.81	1.00	17.14 \pm 2.44	1.00
Liver	12.21 \pm 1.54	0.89	15.54 \pm 2.05	0.79	15.33 \pm 2.75	1.12
Spleen	25.88 \pm 4.78	0.42	21.43 \pm 3.52	0.58	15.43 \pm 3.84	1.11
Kidney	4.65 \pm 0.46	2.34	5.18 \pm 1.04	2.38	4.96 \pm 1.89	3.46
Lung	1.74 \pm 0.24	6.24	1.90 \pm 0.37	6.36	2.67 \pm 0.83	6.42
Heart	0.99 \pm 0.11	10.97	1.18 \pm 0.34	10.45	1.20 \pm 0.55	14.28
Muscle	0.41 \pm 0.07	26.49	0.44 \pm 0.09	28.02	0.49 \pm 0.19	34.98
Blood	1.55 \pm 0.12	7.01	1.99 \pm 0.45	6.20	3.00 \pm 1.37	5.71

^a Each value represents the mean \pm s.d. for six to seven animals.

TABLE 4
Tissue Distribution of ^{14}C radioactivity at 24 hr After the Administration of Liposomes Composed of Various Phospholipids and Cholesterol (Molar Ratio 2:1) and Labeled with Cholesteryl [$1\text{-}^{14}\text{C}$]Oleate*

Tissue	% Administered dose/g					
	PC liposomes	DMPC liposomes	DPPC liposomes	DSPC liposomes	DAPC liposomes	SM liposomes
Tumor	3.11 \pm 0.54	2.81 \pm 0.60	3.84 \pm 0.83	5.85 \pm 0.87	6.42 \pm 0.75	13.05 \pm 2.12
Liver	2.11 \pm 0.53	2.77 \pm 0.61	2.82 \pm 0.59	3.83 \pm 0.32	4.56 \pm 0.71	5.11 \pm 0.98
Spleen	1.16 \pm 0.15	3.09 \pm 0.63	3.49 \pm 0.35	4.35 \pm 0.76	4.54 \pm 0.45	4.23 \pm 1.34
Kidney	0.83 \pm 0.23	0.70 \pm 0.06	0.87 \pm 0.15	2.43 \pm 0.44	3.01 \pm 0.37	3.55 \pm 0.53
Lung	0.81 \pm 0.21	0.67 \pm 0.14	0.70 \pm 0.20	1.55 \pm 0.27	1.80 \pm 0.32	2.38 \pm 0.60
Heart	0.66 \pm 0.20	0.79 \pm 0.23	0.81 \pm 0.14	0.75 \pm 0.48	1.36 \pm 0.29	1.14 \pm 0.46
Muscle	0.29 \pm 0.08	0.26 \pm 0.81	0.29 \pm 0.13	0.39 \pm 0.09	0.46 \pm 0.33	0.50 \pm 0.24
Blood	0.66 \pm 0.20	0.21 \pm 0.07	0.54 \pm 0.18	1.68 \pm 0.27	2.28 \pm 0.43	2.87 \pm 0.41

* Each value represents the mean \pm s.d. for five animals.

liposomes and SM-liposomes completely retained ^{67}Ga during a 48 hr incubation with PBS and retained greater than 90% when incubated with serum.

DISCUSSION

In this paper we report that ^{67}Ga encapsulated in liposomes prepared from DSPC, DAPC, and SM with CH (phospholipid:CH molar ratio, 2:1) could be delivered effectively to the tumor site. ^{67}Ga accumulation in the tumor after i.v. administration of these liposomes was 12–17% AD/g at the peak (Fig. 1). Gallium-67 accumulation in the tumor after i.v. injection of [^{67}Ga] citrate, which is clinically used as a tumor-imaging agent, or free [^{67}Ga]NTA was only ~4% AD/g under the same conditions (Table 3 and 9). Therefore, it is clear that liposomes can be an excellent tool to accumulate ^{67}Ga in the tumor, provided that a suitable lipid composition is selected.

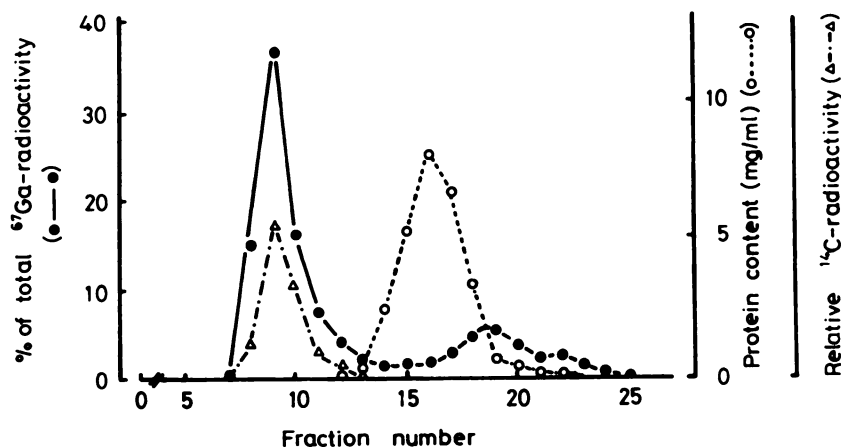
For tumor imaging, the tumor-to-blood ratio of radioactivity accumulation (T/B) is also an important factor. Table 1 shows the sequential changes of T/B after the administration of various liposomes encapsulating [^{67}Ga]NTA. As blood retention of ^{67}Ga with DSPC-, DAPC-, and SM-liposomes was longer than that with other liposomes, their T/B was lower at early stages. However, these liposomes gave excellent T/B at and after 24 hr, owing to their larger tumor accumulation. Further, these liposomes also gave prominent TI. T/B and TI indicate that the optimal imaging time by DSPC-, DAPC-, and SM-liposomes would be 24 hr or 48 hr after the administration, under the condition used in this study. At 24 hr after the administration, these liposomes also exhibit excellent ^{67}Ga accumulation ratios of tumor to the other tissues, except liver and spleen (Table 2). These results suggest that liposomes have great potential as tumor-imaging agents. Reducing the ^{67}Ga accumulation in the liver and spleen will require further work.

The question next arises whether such a high ^{67}Ga accumulation in the tumor reflects the amounts of liposomes themselves that reach the tumor. Gallium-67 itself tends to accumulate in the tumor, as is well known. Thus, we labeled the liposomal lipid phase and traced it. CH was a common component of the liposomes used in this study, but it is known that CH can transfer easily to other liposomes or biomembranes. Thus, we used the ester form of CH, cholesteryl [$1\text{-}^{14}\text{C}$] oleate, because the ester forms hardly transfer to other membranes. Kirby et al. (16) reported that cholesteryl oleate would be a valid marker for liposomes, if the liposomes contain a certain amount of CH. As shown in Table 4, there were significant differences in the tumor accumulations of ^{14}C radioactivity from cholesteryl [$1\text{-}^{14}\text{C}$]oleate at 24 hr after the administration of various liposomes. Much larger values were obtained when DSPC-, DAPC-, and SM-liposomes were administered. This means that these liposomes themselves reached the tumor more effectively than PC-, DMPC- and DPPC-liposomes. Therefore, it is suggested that the larger tumor accumulations of ^{67}Ga observed when DSPC-, DAPC-, and SM-liposomes encapsulating [^{67}Ga]NTA were administered (as shown in Figure 1 and Table 2) results from the larger amounts of these liposomes themselves that reach the tumor.

The tissue distribution rate (% AD/g of tumor) of ^{14}C radioactivity did not necessarily agree with that of ^{67}Ga at this point (Table 2 and 4). The ^{67}Ga radioactivity was accumulated more extensively in the spleen, liver, and tumor than ^{14}C activity, with all of the liposomes. The discrepancy was also observed in the other tissues with PC-, DMPC-, and DPPC-liposomes. If the solutes are retained completely in liposomes, such differences should not be found. Therefore, it would appear that encapsulated [^{67}Ga]NTA was released from liposomes at least partially somewhere in the body, for example in the tumor, liver, spleen or blood. Released [^{67}Ga] NTA may account for a portion of the ^{67}Ga tumor

FIGURE 2

Chromatographic separation of liposome-encapsulated ^{67}Ga and leaked ^{67}Ga on a Sepharose CL-4B column (0.8×25 cm) after incubation with serum at 37°C .



uptake after the administration of ^{67}Ga encapsulated in liposomes, as ^{67}Ga has certain affinity for the tumor (17). However, it is clear that the extensive ^{67}Ga accumulation in the tumor is mainly attributable to the use of liposomes, because the tumor accumulation of ^{67}Ga encapsulated in liposomes was related to the tumor accumulation of the liposomes themselves (Figure 1, Table 2 and 4), and such large values could not be obtained with free ^{67}Ga NTA (Table 3 and 9). We are

currently studying the behavior of ^{67}Ga NTA encapsulated in liposomes and the liposomes themselves in detail.

The question arises, why do DSPC-, DAPC-, and SM-liposomes reach the tumor more effectively than PC-, DMPC-, and DPPC-liposomes? It has been reported that the lipid compositions of liposomes influence their bilayer stability in the presence of serum in vitro and in vivo (12,18-21). Destabilization of lipo-

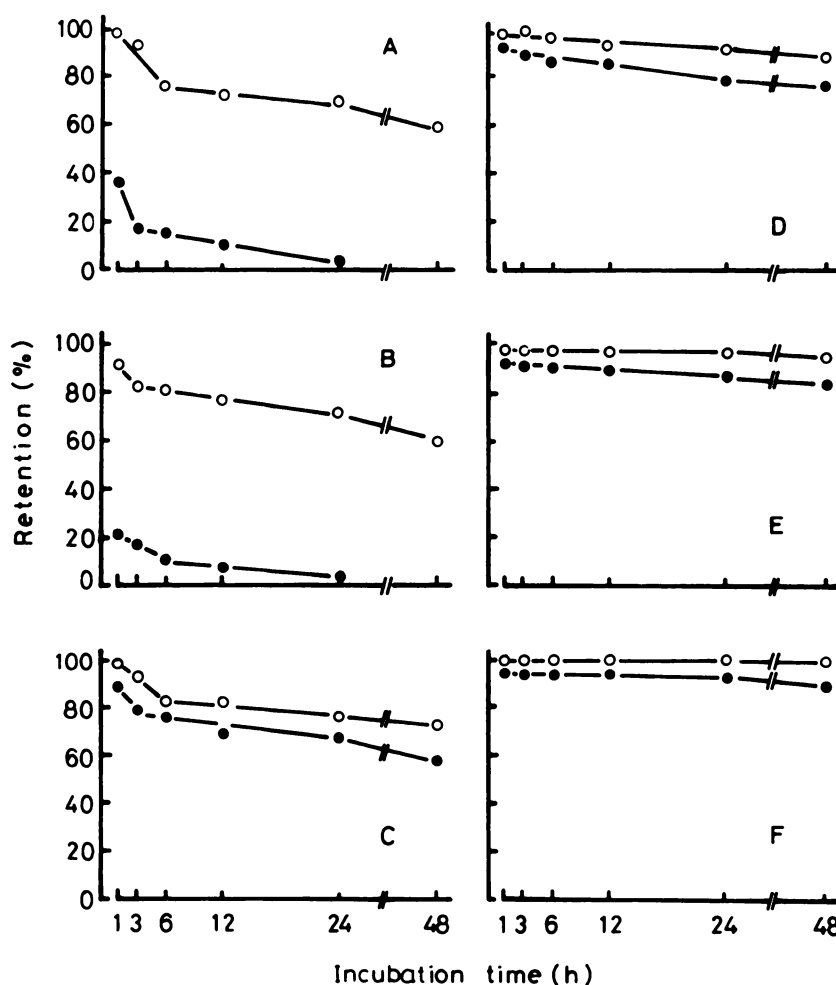


FIGURE 3

Stability of ^{67}Ga -encapsulating liposomes composed of various phospholipids with cholesterol in serum at 37°C in vitro. Liposomes composed of PC (A), DMPC (B), DPPC (C), DSPC (D), DAPC (E), and SM (F) with CH (molar ratio of phospholipid:CH, 2:1) were incubated in PBS (○) or mouse serum (●).

somes, that is, solute leakage through the liposomal bilayers in the serum, has been attributed to the loss of liposomal phospholipids to plasma high density lipoproteins (HDL) (15,22,23). The extent of HDL attack on liposomal membranes is determined predominantly by the membrane fluidity and bilayer packing. Liposomal phospholipid components and the cholesterol content of the membrane decide these two factors (12), and consequently liposome stability.

It is also known that liposome stability in the serum has a close relationship with liposome clearance from the circulation after i.v. injection (12,24,25). More stable liposomes have longer half-lives in the blood circulation.

We determined the stability of the liposomes used in this study in terms of the leakage of ^{67}Ga radioactivity (Fig. 3), and found that DSPC-, DAPC- and SM-liposomes are significantly more stable in the serum than PC-, DMPC- and DPPC-liposomes. The former are taken up by the tumor effectively and deliver large amount of ^{67}Ga to the tumor. We hypothesize that DSPC-, DAPC- and SM-liposomes are resistant to HDL attack in the serum owing to the nature of their phospholipid components, and, when injected intravenously, they remain in the blood circulation for a long time with retention of their integrity. Consequently, they have a greater opportunity to come into contact with the tumor and to be taken up by the tumor, resulting in greater ^{67}Ga accumulation in the tumor. Though DPPC-liposomes were less stable than these three liposomes, they were more stable than PC- or DMPC-liposomes. However, the tumor uptake and ^{67}Ga delivery potential of PC-, DMPC-, and DPPC-liposomes were similar. We consider that fairly stable liposomes are required for increased tumor uptake and ^{67}Ga delivery potential.

We have shown here that some liposomes can be accumulated in the tumor in large quantities and can deliver large amounts of ^{67}Ga to the tumor, provided that a correct choice of the lipid composition is made. We have also suggested that the stability of liposomes in the serum is a critical factor determining the tumor uptake of liposomes and ^{67}Ga delivery to the tumor by the liposomes. Recently several attempts to enhance the stability of liposomes by means of structurally modifying their phospholipids or cholesterol components, or using other lipids instead of phosphatidylcholine and SM, have been reported (19,25-27). We consider that there are excellent prospects for enhancing ^{67}Ga delivery to the tumor further by using such liposomes.

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