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Effects of the size and fluidity of liposomes on their accumulation in tumors: A presumption of their interaction with tumors

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Abstract

The distribution of liposomes with different membrane fluidity and vesicle size in tumors after intravenous injection was investigated in Yoshida sarcoma-bearing rats. Liposomes composed of egg phosphatidylcholine (EPC) or hydrogenated egg phosphatidylcholine (HEPC), dicetyl phosphate and cholesterol in a molar ratio of 5:1:4 were prepared. Their size was adjusted so that they had various mean diameters, ranging from 40 to 400 nm. In EPC liposomes, whose membranes were more fluid than those of HEPC liposomes, tumor accumulation increased with increasing area under the blood concentration-time curve (AUC). The size of liposomes which showed the greatest tumor accumulation and AUC was around 100 nm in diameter. In HEPC liposomes, the less fluid type, the size dependence of tumor accumulation and AUC differed. The greatest tumor accumulation or AUC were found in liposomes with a diameter of about 100 or 40 nm, respectively. This discrepancy indicates that the tumor accumulation of liposomes is not always correlated with their circulation time in the blood. To clarify the process by which these liposomes accumulate from the vascular space into the tumor, we calculated tumor uptake clearance (CL_{tu}), which can separate the contribution of the blood concentration from the accumulation in tumor. The CL_{tu} values for EPC and HEPC liposomes agreed at all sizes, liposomes with a diameter of 100 nm showing the highest values. These findings indicate that the accumulation of liposomes from the vascular space into the tumor is primarily governed by their size and not by their membrane fluidity or blood circulation time. When tumor blood flow was selectively enhanced by the infusion of angiotensin II, the CL_{tu} of 100-nm liposomes decreased to the level of that in 40-nm liposomes, suggesting that some histological factor(s) in the tumor may be responsible for the localization of 100-nm liposomes in tumor. In an in vitro experiment using cultured Yoshida sarcoma cells, 59-nm HEPC liposomes were directly taken up by the tumor cells to an extent at least 2.5-times greater than larger liposomes ($\geq$ 100 nm). We conclude that 100-nm liposomes may predominantly localize in the interstitial space, whereas some liposomes of smaller size may be taken up by tumor cells.

Keywords: Liposome; AUC; Tumor uptake clearance; Angiotensin II; Yoshida sarcoma

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1. Introduction

Although liposomes have been extensively investigated as drug carriers for delivery to tumors, there are only a few basic studies on their accumulation in tumors. A number of groups (Gabizon and Papahadjopoulos, 1988; Liu et al., 1992) have recently reported that the optimal size of liposomes for maximizing their accumulation in tumors is around 100 nm diameter. These investigators have emphasized that the tumor accumulation of liposomes depends on their circulation time in the blood. However, the findings of these studies did not show the actual accumulation of liposomes from the blood space into the tumor, since their accumulation in the tumor is a result of three factors: their concentration in the blood, their blood-to-tumor transfer, and their interaction with the interstitial or tumor cells. To clarify the mechanism by which liposomes accumulate in tumor, it would be necessary to investigate these processes.

The circulating time and amount of liposomes in the blood vary depending on their uptake by cells in the reticuloendothelial system (RES), such as macrophages in the liver and spleen (Senior et al., 1992; Allen and Chonn, 1987). Thus, the contribution of liposome concentration in blood must be separated from the amount of tumor accumulation to evaluate accurately their distribution process from the vascular space to tumor. Tumor uptake clearance (CL_{tu}) can be used as a parameter that separates the contribution of blood concentration from tumor accumulation, since it is defined as a quotient of the tumor accumulation divided by the area under the blood concentration-time curve (AUC). Such an evaluation with CL_{tu} may provide important information about the accumulation process of liposomes from vascular to interstitial spaces.

In vivo pharmacokinetic studies provide no information on the interaction of liposomes with tumor cells. Several investigators (Papahadjopoulos et al., 1991; Huang et al., 1992) have confirmed microscopically the extravasation of liposomes with a diameter of about 100 nm into tumor interstitial spaces and the less likely possibility that they are directly taken up by tumor cells. Although microscopic studies are useful for clarifying the localization of liposomes in tumor at the cellular or subcellular level, in vitro cellular uptake investigations may be necessary for obtaining more detailed information on the interaction of liposomes with tumor cells.

In this study, the size dependence of CL_{tu} and the amount of tumor accumulation were compared in liposomes with different fluidity. The use of these liposomes clarifies the effect of separating the contribution of liposome concentration in blood, since this fluidity affects their blood concentration by changing their RES uptake (Patel, 1992). We also examined the effect of tumor blood flow on CL_{tu} following the infusion of angiotensin II, which selectively increases tumor blood flow (Suzuki et al., 1981), to elucidate the involvement of tumor structural factor(s) in the localization of liposomes in a tumor. We also studied the effect of the size of liposomes on their interaction with tumor cells in vitro. On the basis of the findings in these studies, we suggest a mechanism that explains the localization of liposomes in tumors.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (EPC) and hydrogenated egg phosphatidylcholine (HEPC) were kindly supplied by Nippon Oil and Fats Co., Ltd (Tokyo, Japan) and Nippon Fine Chemical Co., Ltd (Osaka, Japan), respectively. Dicetyl phosphate (DCP) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Cholesterol (Ch) and angiotensin II were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). [^{3}H]Cholesteryl hexadecyl ether was obtained from NEN (Boston, MA). Other chemicals used were of reagent grade.

2.2. Liposome preparation

Liposomes composed of EPC or HEPC, DCP and Ch in a molar ratio of 5:1:4 were prepared as reported previously (Harashima et al., 1992) with

a minor modification. In brief, the lipids were dissolved in chloroform with a trace of [^{3}H]cholesteryl hexadecyl ether as a lipid phase marker and the solvent was evaporated in vacuo. The resulting lipid films (200 μmol as total lipids) were hydrated in 5 ml of pH 7.4 isotonic phosphate buffer (Na_2HPO_4, 1.15 g; KH_2PO_4, 0.2 g; NaCl, 8 g; KCl, 0.2 g/l) at 40–45° C. The liposomal suspensions were extruded through a polycarbonate membrane filter (Nuclepore Co., Pleasanton, CA) with a pore size of 0.4, 0.2 or 0.1 μm at room temperature. Small unilamellar vesicles (SUVs) were prepared by using an ultrasonication technique with a probe-type sonicator (UR-200P, Tomy, Tokyo, Japan) until the lipid suspension became transparent for about 90 min on ice. The SUVs were centrifuged at $100\,000 \times g$ for 60 min to remove large particles and to provide a homogeneous size. Liposome size was determined by dynamic light scattering with a submicron analyzer (Nicomp model 370, Nicomp Particle Sizing Systems, Santa Barbara, CA).

2.3. Biodistribution of liposomes

Male Donryu rats weighing 160–180 g (SLC, Shizuoka, Japan) were subcutaneously inoculated in the back with 5×10^6 Yoshida sarcoma cells. The animals were used for experiments 4 or 5 days after inoculation. Liposomes were intravenously injected via the tail vein at a dose of 100 μmol lipid/kg. After a specified period, blood samples were taken from the abdominal vein and the animals were killed by dislocation of the abdominal vein and artery after they had been anesthetized with diethyl ether. Liver, spleen, lung, kidney, muscle and tumor were excised and weighed. After decolorization with 35% H_2O_2 and dissolution with 2 M KOH in isopropyl alcohol (Harashima et al., 1992), the radioactivity in each sample was measured with an Aloka LSC-700 liquid scintillation counter. The weight of rat blood was assumed to be 6.5% of the body weight (Tröser et al., 1992). The area under the blood concentration-time curve (AUC) was calculated according to the trapezoidal rule. Tumor uptake clearance (CL_{tu}) was calculated by dividing the amount of liposomes accumulated in the tumor by the corresponding AUC.

2.4. Elevation of tumor blood flow by angiotensin II infusion

Catheters were introduced into the femoral vein and artery of tumor-bearing rats for angiotensin II infusion and blood sampling, respectively. Angiotensin II was infused for 15 min, at a rate of 1 g/kg/min, through the catheter, and liposomes were injected through the catheter into the femoral vein. The infusion of angiotensin II was continued for 4 h after the injection of liposomes. Blood samples were collected through the arterial catheter at appropriate intervals up to 4 h after the injection of liposomes. Immediately after the angiotensin II infusion was stopped, the rats were killed as described above and the tumors were excised. In the control group, 0.9% NaCl solution was infused instead of angiotensin II. CL_{tu} was calculated as described above.

2.5. Incubation of liposomes with tumor cells

A primary culture of Yoshida sarcoma cells was used. The cell culture medium was prepared by supplementing RPMI 1640 medium (Nissui Co., Ltd, Tokyo, Japan) with 10% heat-inactivated fetal calf serum (Gibco, Grand Island, NY). Tumor cells, taken from male Donryu rats bearing 4-day-old Yoshida sarcoma ascites, were cultivated in culture dishes at 4×10^5 cells/well overnight at 37° C in a humidified atmosphere of 5% CO_2 in air. Liposomes (500 nmol as total lipids) were added to each test well. After incubation at 37 or 4° C for 24 h, the resuspended cells were collected by centrifugation (1000 rpm, 5 min, 4° C) and washed twice with cold pH 7.4 isotonic phosphate buffer. The cells were then dissolved in 0.5 ml of 1 M NaOH and neutralized with 0.5 ml of 1 M HCl. The radioactivity in each sample was measured with an Aloka LSC-700 liquid scintillation counter.

3. Results

3.1. Effects of membrane fluidity of liposomes on their blood circulation and accumulation in tumor

Fig. 1 shows the time courses of the blood levels and liver uptake of saturated PC (HEPC)

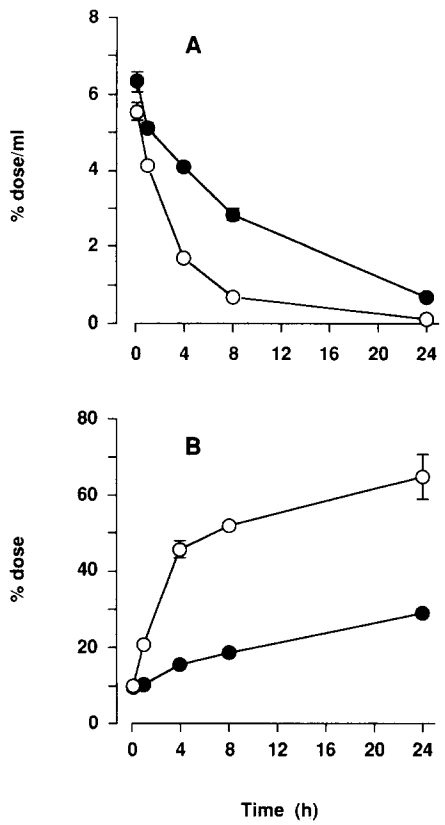

Fig. 1. Effect of membrane fluidity on blood concentration (A) and liver accumulation (B) of small unilamellar liposomes with a diameter of about 40 nm after intravenous injection into Yoshida sarcoma-bearing rats. ^{3}H-labeled liposomes were injected at a dose of 100 μmol lipid/kg into rats bearing Yoshida sarcoma. After blood samples were taken via the abdominal vein at the indicated time, organs were excised and weighed. Lipid composition (molar ratio): (●) HEPC/DCP/Ch (5:1:4); (○) EPC/DCP/Ch (5:1:4). Each point represents the mean ± S.E. of four rats.

and unsaturated PC (EPC)-based liposomes (HEPC and EPC liposomes, respectively) with a diameter of about 40 nm in Yoshida sarcoma-bearing rats. The membrane fluidity of HEPC liposomes has been confirmed to be less than that of EPC liposomes by the fluorescence polarization method (Nagayasu et al., 1995). HEPC liposomes exhibited a prolonged blood circulation time and decreased liver uptake compared with the EPC liposomes. Values for tumor accumulation, uptake by RES (liver and spleen) and AUC until 24 h after injection of these liposomes are summarized in Table 1. The tumor accumulation of 40-nm HEPC liposomes was markedly greater than that of 40-nm EPC liposomes. This accumulation corresponded to their AUC. Further, the amounts of 40-nm HEPC and EPC liposomes accumulated in tumors were 50- and 30-fold greater, respectively, than those in muscle (data not shown).

3.2. Effects of liposome size on AUC and RES uptake of liposomes

Fig. 2 demonstrates the effect of liposome size on the AUC of HEPC and EPC liposomes. The AUC increased with decrease in the size of HEPC liposomes, whereas it showed maximal value at 134 nm diameter for EPC liposomes. There was no obvious difference in the AUC between HEPC and EPC liposomes with diameters of more than 200 nm. As shown in Table 2, the size dependence of RES uptake was opposite to that of the AUC for both types of liposomes.

Table 1
Effects of membrane fluidity on tumor accumulation, AUC and RES uptake of liposomes with a diameter of about 40 nm 24 h after intravenous injection into rats bearing Yoshida sarcoma

Lipid composition (molar ratio)	Tumor accumulation (% dose/g)	AUC_{0-24} (% dose h) [a]	RES uptake (% dose) [b]
HEPC/DCP/Ch (5:1:4)	4.0 (1.0)	61.7	35.9 (2.3)
EPC/DCP/Ch (5:1:4)	1.7 (0.5)	24.6	68.4 (11.6)

^{3}H-labeled liposomes were intravenously injected at a dose of 100 μmol lipid/kg into rats bearing Yoshida sarcoma; values represent the means (SE) of four rats.

[a] AUC was estimated from the blood concentration-time curve from 0 to 24 h by the trapezoidal rule.

[b] RES uptake represents the total amount of liposomes taken up by the liver and spleen up to 24 h after intravenous injection.

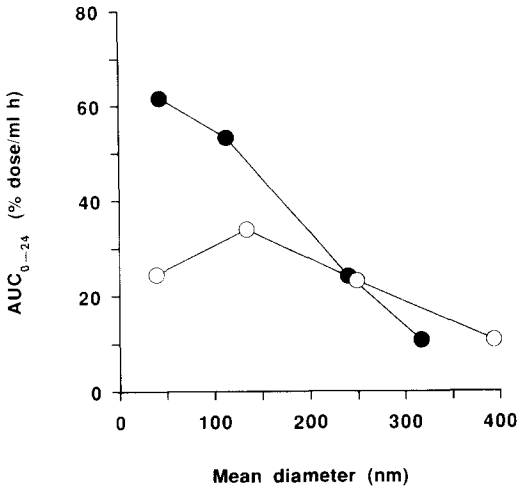

Fig. 2. Effects of size and membrane fluidity on AUC of liposomes in tumor-bearing rats. ^{3}H-labeled liposomes were injected at a dose of 100 μmol lipid/kg into rats bearing Yoshida sarcoma. Blood samples were taken via the abdominal vein at the indicated time. AUC was estimated from blood concentration curves from 0 to 24 h by the trapezoidal rule. Symbols as in Fig. 1.

Table 2
Effects of size and membrane fluidity on RES uptake of liposomes 24 h after intravenous injection into rats bearing Yoshida sarcoma

Lipid composition (molar ratio)	Size (nm)	RES uptake (% dose) [a]
HEPC/DCP/Ch (5:1:4)	43	35.9 (2.3)
	113	49.9 (0.7)
	240	74.2 (3.3)
	317	73.7 (6.3)
EPC/DCP/Ch (5:1:4)	39	68.4 (11.6)
	134	52.2 (8.1)
	249	75.6 (17.5)
	394	74.8 (7.4)

^{3}H-labeled liposomes were intravenously injected at a dose of 100 μmol lipid/kg into rats bearing Yoshida sarcoma; values represent the means (SE) of four rats.
[a] RES uptake represents the total amount of liposomes taken up by the liver and spleen up to 24 h after intravenous injection.

3.3. Effects of liposome size on tumor accumulation and tumor uptake clearance (CL_{tu}) of liposomes

Fig. 3 shows the tumor accumulation of HEPC and EPC liposomes with various average diameters 24 h after intravenous injection. The tumor accumulation of HEPC liposomes was greater for diameters of less than 200 nm, maximal accumulation occurring at diameters of around 100 nm. The tumor accumulation of EPC liposomes showed the same size dependence as that of HEPC liposomes, although the EPC liposomes displayed lower tumor levels at diameters of about 100 nm or less compared with HEPC liposomes. There was no significant difference in tumor accumulation between HEPC and EPC liposomes of more than 200 nm diameter. Interestingly, the size dependence of tumor accumulation in EPC liposomes was similar to that of the corresponding AUC, while the size dependence of HEPC liposomes was different from that of the corresponding AUC (Fig. 2). Fig. 4 shows the size dependence of CL_{tu} for HEPC and EPC liposomes. No obvious difference in this parameter was found between HEPC and EPC liposomes. The CL_{tu} value was highest at around 100 nm for both types of liposomes.

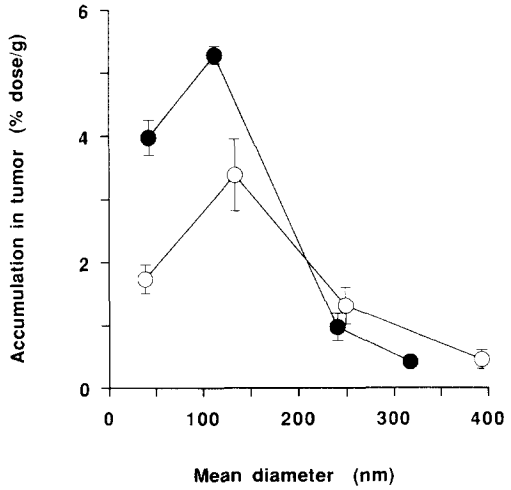

Fig. 3. Effects of size and membrane fluidity on tumor accumulation of liposomes. ^{3}H-labeled liposomes were injected at a dose of 100 μmol lipid/kg into rats bearing Yoshida sarcoma. Rats were killed 24 h after injection, and then tumors were excised and weighed. Symbols as in Fig. 1. Each point represents the mean ± S.E. of at least three rats.

3.4. Effect of changes in blood flow on tumor uptake clearance (CL_{tu})

It is still uncertain where the accumulated liposomes localize in tumor tissue in vivo. We speculated that the size-dependent CL_{tu} (Fig. 4) may be related to the structure of blood vessels in the tumor. The hypertension induced by angiotensin II is known to selectively increase blood flow in tumors due to the absence of blood flow autoregulation in tumors (Pálvölgyi, 1969). Such an increase in local blood flow could affect the permeation of liposomes through tumor capillaries. Fig. 5 shows the effect of angiotensin II on size-dependent CL_{tu} in HEPC liposomes. A continuous infusion of angiotensin II into tumor-bearing rats decreased the CL_{tu} of liposomes around 100 nm in diameter to the level for those of 40 nm, which did not change. The CL_{tu} of larger liposomes with a diameter of more than 200 nm was virtually unchanged.

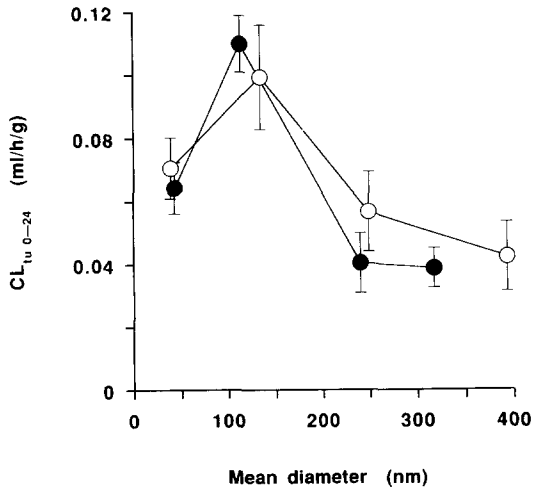

Fig. 4. Size-dependent tumor uptake clearance (CL_{tu}) of liposomes. ^{3}H-labeled liposomes were injected at a dose of 100 μmol lipid/kg into rats bearing Yoshida sarcoma. Rats were killed 24 h after injection, and then tumors were excised and weighed. CL_{tu} was calculated by dividing the amount of liposomes accumulated in the tumor by the corresponding AUC. Symbols as in Fig. 1. Data represent the mean ± S.E. of at least three rats.

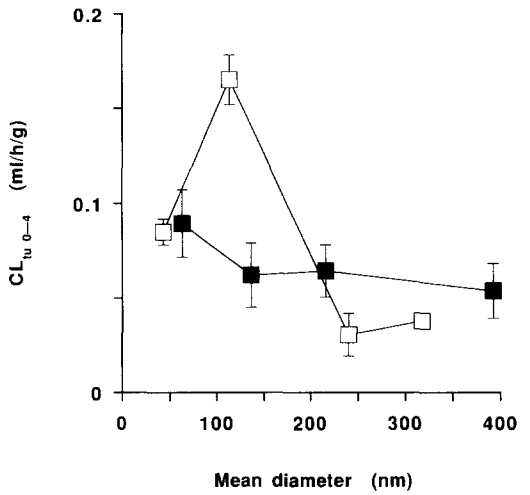

Fig. 5. Effects on angiotensin II on tumor uptake clearance (CL_{tu}) of liposomes composed of HEPC/DCP/Ch (5:1:4). After infusion of angiotensin II (■) or 0.9% NaCl solution (□) for 15 min into rats subcutaneously inoculated with Yoshida sarcoma cells, ^{3}H-labeled liposomes were injected at a dose of 100 μmol lipid/kg into rats bearing Yoshida sarcoma and the infusion of angiotensin II or 0.9% NaCl solution was continued for 4 h. Rats were killed 4 h after injection, and then tumors were excised and weighed. CL_{tu} was calculated as described in Fig. 4. Data represent the mean ± S.E. of at least three rats.

3.5. Uptake of liposomes by cultured tumor cells in vitro

Fig. 6 shows the uptake of HEPC liposomes of various average diameters by cultured Yoshida sarcoma cells. The cellular uptake of 59-nm liposomes was at least 2.5-fold more than that of larger liposomes (116, 283 and 331 nm) at 37° C. The greater uptake of smaller liposomes was not found on incubation at 4° C. The size dependence of the direct cellular uptake of HEPC liposomes at 37° C did not correspond to that of their tumor accumulation or CL_{tu}, as shown in Fig. 3 and 4.

4. Discussion

In tumors, capillary permeability is enhanced and the lymphoid system is little developed compared with normal tissue (Devorak et al., 1988). This morphology suggests that small liposomes

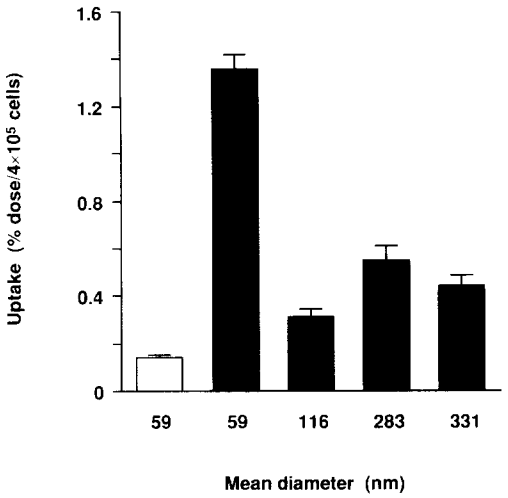

Fig. 6. In vitro cellular uptake of liposomes composed of HEPC/DCP/Ch (5:1:4) by cultured Yoshida sarcoma cells. Yoshida sarcoma cells were incubated with liposomal suspensions at 37° C (filled bar) or 4° C (empty bar) for 24 h. Data represent the mean ± S.E. of four experiments.

may extravasate more easily into extracellular spaces around tumors through the leaky capillaries. Prolonging the circulation time in the blood of small liposomes will increase their chance of encountering leaky vessels. The optimal size of liposomes for elevating their accumulation in tumor was recently reported to be 90–200 nm in diameter (Liu et al., 1992). The latter authors found that liposomes smaller than this size range showed less accumulation in the tumor because the amount of circulating liposomes in the blood was decreased, this being attributed to increased uptake by hepatic parenchymal cells, since the fenestration in liver sinusoids contains holes with an average diameter of 100 nm. Our findings for EPC liposomes agree with those of Liu et al. (Fig. 2, 3 and Table 2). However, there was a discrepancy in the findings for HEPC liposomes, since 40-nm HEPC liposomes, which had the longest residence time in the blood, showed inferior tumor accumulation to the 100-nm liposomes. This finding indicates that the tumor accumulation of liposomes is not always correlated with their circulation time in the blood, and that circulation time may be a secondary factor in determining the amount of liposomes accumulated in the tumor in some cases.

The highest AUC being for 40-nm HEPC liposomes may be due to their small size and lower membrane fluidity. In general, smaller liposomes are taken up less by the RES. The lower membrane fluidity may lead to decreased recognition by both hepatic parenchymal cells and non-parenchymal cells, such as Kupffer cells, due to reduced interaction with blood component(s) (Allen and Chonn, 1987; Patel, 1992; Senior et al., 1992).

We selected the CL_{tu} to evaluate correctly the process by which liposomes accumulate from the blood space into the tumor, since this parameter separates liposome concentration in blood from the amount accumulated in the tumor. The agreement of CL_{tu} for HEPC and EPC liposomes at all sizes and the maximal values of these liposomes with diameters of 100 nm (Fig. 4) indicate that the accumulation of liposomes from the vascular space into the tumor is primarily governed by their size, and not by their membrane fluidity or circulation time in the blood.

This is considered to be applied to liposomes without any surface modification, since the change in the affinity of liposomes for blood components and tumor cells by modifying their membranes with ligands to targeted sites, such as antibodies or galactose, may show greater effect on their accumulation from the vascular space into the tumor than their size.

Although the mechanism of transfer of liposomes from vascular to extracellular spaces around tumors is poorly understood, two possibilities have been considered: their permeation through leaky capillary structures such as fenestrated endothelium or capillary sprouts, and their transendothelial vesicular transport (Huang et al., 1992). The latter represents an important means of transport for smaller liposomes with a diameter of about 40 nm, as their size is comparable with that of endosomes (Steinman et al., 1976). However, this transcytosis of liposomes by endothelial cells may not be an important factor, since no saturation of tumor uptake was observed at a 4-fold higher dose than the present dose with 40-nm HEPC liposomes (data not shown). Thus, the passage of liposomes through leaky capillaries appears to be the more likely mechanism.

The accumulation of liposomes in tumor is a result of their influx and efflux between the vascular space and the tumor. Thus, the maximal CL_{tu} of 100-nm liposomes shows that their efflux into the blood may be more limited by tumor tissue compared with those of 40 nm. Since the lymphatic drainage system is little developed in tumors (Devorak et al., 1988), the decrease in CL_{tu} of only 100-nm liposomes by the infusion of angiotensin II implies an increase in their efflux into the blood and no change in that of smaller (40 nm) or larger ($\geq$ 200 nm) liposomes. For 100-nm liposomes, factor(s) other than passage through leaky capillaries may also be responsible for their accumulation in the tumor. Smaller liposomes (40 nm) may show fairly free transfer between the vascular space and the tumor. This is supported by microscopic observations that the capillary endothelium in a certain brain tumor (Hirano and Matsui, 1975) and Walker 256 carcinoma (Warren, 1970) contains pores of 50 nm in diameter. However, larger liposomes ($\geq$ 200 nm) would be unlikely to pass through leaky capillaries even when angiotensin II infusion is performed.

The results in Fig. 6 shows that smaller liposomes with a diameter of about 50 nm can be directly taken up by tumor cells to an extent 4-fold greater than that in the case of 100-nm liposomes. It is possible that the marked uptake of smaller liposomes is due to endocytosis which is enhanced in tumor cells (Guminska and Kieler, 1967), since these liposomes are as small as endosomes (Steinman et al., 1976) and their uptake was significantly inhibited at 4° C. These findings and those of the CL_{tu} (Fig. 4 and 5) suggest that most 100-nm liposomes may remain in the interstitial spaces without uptake by tumor cells, while some smaller liposomes may be taken up by these cells, after they have accumulated in tumor tissue.

The size of 100 nm is thought to be suitable not only for the permeation of liposomes through leaky capillaries, but also for their localization in the interstitial spaces. The extravasation of liposomes of this size into the extracellular spaces surrounding capillary endothelial cells in tumors was recently confirmed (Huang et al., 1992). The difference between 40- and 100-nm liposomes in local residence in tumor tissue may be due to their interaction with the tissue (Fig. 7, left). Tumors have large interstitial spaces composed predominantly of the collagen and elastic fiber network (Jain, 1989). Liposomes with a diameter of 100 nm may be more readily trapped by the fiber network and the leakiness of capillary walls may be insufficient to allow their efflux from the interstitial into the vascular space. Their interac-

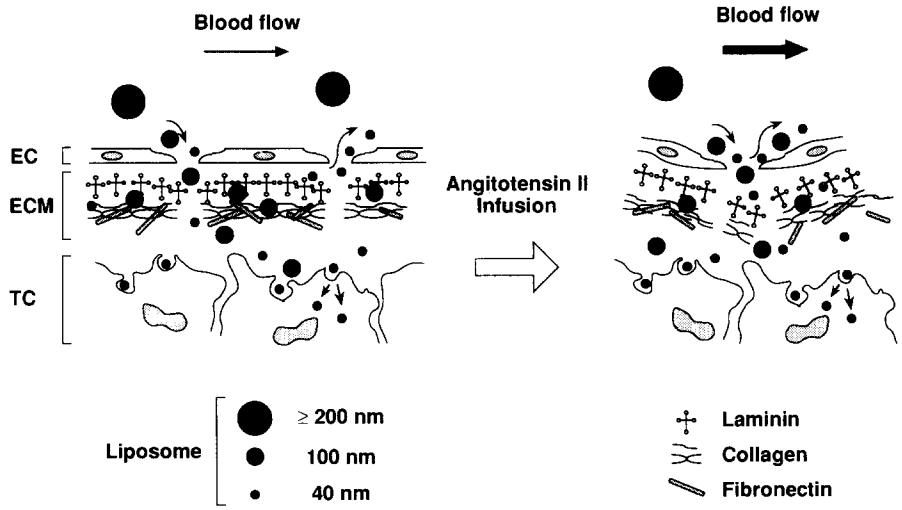

Fig. 7. Schematic diagrams of the interaction of liposomes with tumor in the absence (left) or presence (right) of the infusion of angiotensin II. EC, endothelial cell; ECM, extracellular matrix; TC, tumor cell.

tion with these structures, together with their easy passage through the leaky capillaries, may maximize their accumulation from vascular to interstitial spaces. On the other hand, if 40-nm liposomes are small enough to pass through the leaky capillaries, their efflux from the interstitial to the vascular space may occur more freely than that of 100-nm ones. Some of them in the interstitial space may pass through the fiber network to be taken up by tumor cells via endocytosis.

The interaction of 100-nm liposomes with these components in tumors appears to be decreased by the increased blood flow in the tumor induced by angiotensin II (Fig. 7, right). It is conceivable that the increase in blood flow may enhance both the convection from the vascular to the interstitial space (Jain, 1989) and the leakiness of capillaries. The increase in convection may decrease the trapping of 100-nm liposomes by the fiber network and increase the number of free liposomes near capillaries. The increase in leakiness of capillaries or in the blood flow may facilitate the efflux of 100-nm liposomes into the blood, whereas the free movement of 40-nm liposomes between vascular and interstitial spaces may cause them to be insensitive to increased blood flow in the tumor.

Although we have no obvious evidence of the interaction of liposomes with tumor tissue, the following notable findings were obtained by evaluating the accumulation of liposomes in tumors, using CL_{tu}: the accumulation of liposomes from the vascular space into the tumor is primarily determined not by their concentration in blood, but by their size; it is greatest at about 100 nm diameter. Although 100-nm liposomes have been known to show maximal accumulation in tumor (Gabizon and Papahadjopoulos, 1988; Liu et al., 1992), we have demonstrated the adequacy of this size in the present pharmacokinetic study.

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