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Tissue Distribution and Pharmacokinetic Evaluation of the Targeting Efficiency of Synthetic Alkyl Glycoside Vesicles[1)]

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The tissue distribution of alkyl glycoside vesicles (AGV), which are based on synthetic alkyl glycosides, was examined after intravenous administration in mice, and the results were compared with those for phosphatidylcholine liposomes (PC liposomes). Two types of AGV were used in this study, *i.e.*, vesicles based on stearyl galactoside (Gal-ST vesicles) and stearyl glucoside (Glu-ST vesicles). The Gal-ST vesicles showed significantly higher accumulation in the liver compared with the Glu-ST vesicles and PC liposomes, and this high accumulation was maintained for a long time (8 h). On the other hand, the Gal-ST vesicles and the Glu-ST vesicles showed lower accumulation in the spleen than PC liposomes. It appears that galactoside vesicles have an affinity for the liver and the AGV intrinsically have a lower affinity for the spleen than PC liposomes. Targeting of galactoside vesicles to the liver was expected.

In order to evaluate the tissue distribution profiles quantitatively, pharmacokinetic analysis of the time course data was carried out based on a physiological flow model. It has become apparent in this analysis that the affinity of the Gal-ST vesicles for the liver was as high as 1.5 times those of Glu-ST vesicles and PC liposomes, while those of Gal-ST vesicles and Glu-ST vesicles for the spleen were about a half of that of PC liposomes. It also appears that the degradation rate of these vesicles in the tissues differs with different types of vesicles. Such an analysis leading to quantitative evaluation of the targeting efficiency should be useful for developing vesicles as drug carriers.

Keywords——alkyl glycoside vesicle; liposome; tissue distribution; targeting; pharmacokinetic analysis; simulation; drug carrier; stearyl galactoside; stearyl glucoside

Introduction

In the previous paper,[2)] it was observed that long chain alkyl glycosides form liposome-like vesicles, and their encapsulation capacity and physical properties were reported. The alkyl glycoside vesicles (AGV) showed encapsulation capacity comparable to that of phosphatidylcholine liposomes (PC liposomes) and higher stability in a normal atmosphere. They showed faster release of their encapsulated contents than the PC liposomes during incubation with rat plasma at 37 °C. However, the release decreased gradually and about 60% of the initial content was retained in the vesicles after 48 h of incubation, whereas PC liposomes showed a continuous degradation and the retention of the content after 48 h was only about 30%. Thus, the AGV interacted differently from PC liposomes with plasma components.

On the other hand, Ashwell and Morell[3)] studied the specific receptor for galactose terminating glycoprotein on the surface of hepatocytes, and thereafter targeting to the liver of liposomes which have a galactose residues was attempted.[4)] Recently, Spanjer and Scherphof reported[5)] successful targeting of lactosylceramide-containing liposomes to hepatocytes *in vivo*.

In this study, targeting of galactoside vesicles to the mouse liver was attempted and the tissue distribution after intravenous administration to mice was examined. The results are compared with those for glucoside vesicles and PC liposomes and discussed. Time courses of

the tissue distributions were simulated by using a pharmacokinetic flow model and the targeting effciency of the vesicles was evaluated quantitatively.

Experimental

Materials——Egg L-α-phosphatidylcholine (PC), dicetyl phosphate (DCP), and cholesterol (CH) were obtained as described in the previous paper.[2] Stearyl galactoside (Gal-ST) and stearyl glucoside (Glu-ST) were synthesized as described in the previous paper.[2] D-α-Tocopherol (α-T) was purchased from Sigma Chem. Co. (St. Louis, MO). [^{3}H(G)]Inulin was from New England Nuclear (Boston, MA). All other chemicals were of reagent grade or better.

Preparation of Vesicles——Reverse-phase evaporation vesicles (REV) of PC, Glu-ST, and Gal-ST as a base were prepared as described in the previous paper.[2] They were composed of the base, DCP, and CH in a molar ratio of 4:1:3, but in the case of the PC liposomes, 0.1 eq of α-T was added as an antioxidant to protect egg PC.[6] As an aqueous marker, [^{3}H]inulin was used in this study, because the substance is biologically inert and is excreted rapidly. Therefore, it may be considered that the radioactivity in the tissue or blood reflects the behavior of the vesicles.[10]

The vesicles were extruded through polycarbonate membranes (Nuclepore Co., CA) having pore sizes of 0.8, 0.4, and 0.2 μm, successively,[8] and then dialyzed[9] with a membrane having a pore size of 0.2 μm in a flow-type dialysis cell[10] against 1000 volumes of phosphate-buffered saline (PBS) for sizing of the vesicles and removing unencapsulated aqueous maker. The dialysis was continued at 4 °C for 48 h; the PBS was changed three or four times.

Tissue Distribution Study——A 0.2 ml aliquot of liposomal suspension (0.8 μmol of the base lipid) was injected intravenously *via* the tail vein into DDY male mice (20 g body weight). At appropriate times after injection (0.25, 0.5, 1.0, 4.0, and 8.0 h), mice were sacrificed by puncturing the jugular vein under light anesthesia with ether and the blood was removed rapidly with a heparinized syringe.[7] Liver, lung, spleen (these are reticuloendothelial system (RES)-rich organs) and kidneys (reference organ) were subsequently removed and rinsed with PBS. Whole blood (0.5 ml), a part of the liver (0.2 g), and other organs were transferred into combustion cups and allowed to dry for 2—4 h under an infrared lamp. Radioactivities of the samples were counted with a liquid scintillation counter (Aloka LSC-673) after combustion in a sample oxidizer (Packard serial No. 19925).

Pharmacokinetic Simulation——In order to evaluate the tissue distribution profiles of the vesicles quantitatively, simulation of the time courses was carried out based on a pharmacokinetic flow model. The model used in this study is presented in Fig. 1. This model consists of four organ compartments (lung, liver, spleen, and kidney) and artery and vein compartments, and all organ compartments were divided into a blood compartment and a tissue compartment. The transfer of the vesicles was assumed to occur as shown by arrows in Fig. 1. In the model, the mass valances of the amounts in the compartments were expressed as simultaneous differential equations as follows;

$$\frac{dX_1}{dt}=\frac{X_2}{V_2}\times(Q_t-Q_4-Q_5-Q_6)+\frac{X_4}{V_4}\times(Q_4+Q_5)+\frac{X_6}{V_6}\times Q_6-\frac{X_1}{V_1}\times Q_t-k_e\times X_1$$

$$\frac{dX_2}{dt}=\frac{X_3}{V_3}\times Q_t-\frac{X_2}{V_2}\times Q_t-k_e\times X_2$$

$$\frac{dX_3}{dt}=\frac{X_1}{V_1}\times Q_t-\frac{X_3}{V_3}\times Q_t+k_{73}\times X_7-k_{37}\times X_3$$

$$\frac{dX_4}{dt}=\frac{X_2}{V_2}\times Q_4+k_{84}\times X_8+\frac{X_5}{V_5}\times Q_5-k_{48}\times X_4-\frac{X_4}{V_4}\times(Q_4+Q_5)$$

$$\frac{dX_5}{dt}=\frac{X_2}{V_2}\times Q_5+k_{95}\times X_9-\frac{X_5}{V_5}\times Q_5-k_{59}\times X_5$$

$$\frac{dX_6}{dt}=\frac{X_2}{V_2}\times Q_6+k_{106}\times X_{10}-\frac{X_6}{V_6}\times Q_6-k_{610}\times X_6$$

$$\frac{dX_7}{dt}=k_{37}\times X_3-k_{73}\times X_7$$

$$\frac{dX_8}{dt}=k_{48}\times X_4-k_{84}\times X_8-k_{e8}\times X_8$$

$$\frac{dX_9}{dt}=k_{59}\times X_5-k_{95}\times X_9-k_{e9}\times X_9$$

$$\frac{dX_{10}}{dt}=k_{610}\times X_6-k_{106}\times X_{10}$$

where V_1—V_6 are the blood volume in each compartment, and other symbols are described in Fig. 1. The time courses of the vesicles in the blood and the tissues were simulated by solving these equations numerically with appropriate parameter values. The parameter values of the blood flow (Q_t and Q_4—Q_6) and the volume of blood compartments (V_1—V_6) were obtained by calculation from data found in the literature.[11,12] The degradation rate constants in blood (k_e) were changed slightly on the basis of the values obtained in the *in vitro* stability study reported in the previous paper.[2] The affinity of the vesicles to the tissue was evaluated in terms of the values of the first-order rate constants for the transfer of vesicles from blood to tissue and from tissue to blood compartments.

The computation was carried out on a digital computer (HITAC M-280H) at the Computer Center of the University of Tokyo. The program for numerically solving the differential equations by the Runge–Kutta method was written by the authors in Fortran.

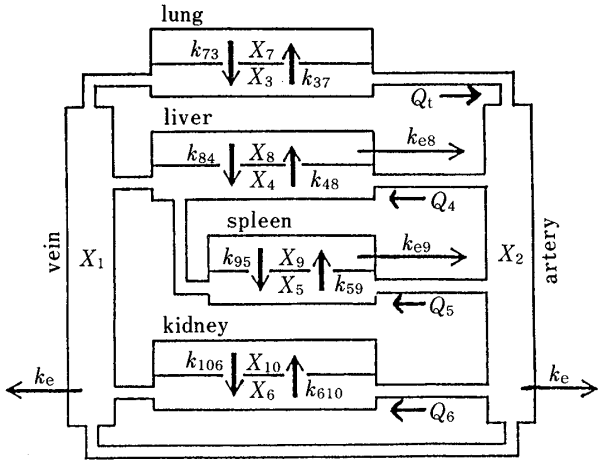

Fig. 1. Pharmacokinetic Model for *in Vivo* Behavior of the Vesicles

k_{37}, k_{48}, k_{59}, and k_{610} are first-order rate constants for the transfer of vesicles from blood to tissue in each organ. k_{73}, k_{84}, k_{95}, and k_{106} are first-order rate constants for the transfer of vesicles from tissue to blood in each organ. k_{e_8} and k_{e_9} are first-order rate constants for the elimination or degradation of vesicles in tissue compartments. k_e is first-order rate constant for the elimination or degradation of vesicles in blood compartment. X_1 and X_2 are the amounts of vesicles in blood compartments (in veins and arteries, respectively). X_3—X_6 are the amounts of vesicles in blood compartments in each organ. X_7—X_{10} are the amounts of vesicles in tissue compartments in each organ. Q_4—Q_6 are blood flow rates through into each organ. Q_t is cardiac blood flow rate.

Results and Discussion

Tissue Distribution

The results are summarized in Table I. The Gal-ST vesicles were found at a lower level in the blood at 15 min after intravenous administration in mice as compared with PC liposomes, but at higher levels than PC liposomes thereafter. Similar results were obtained with Glu-ST vesicles. These blood level characteristics of the AGV may reflect the relative stability in plasma as discussed in the previous report.[2] The AGV showed rapid release in the early stages followed by a plateau level of stability on incubation with plasma at 37 °C.[2]

The Gal-ST vesicles showed higher accumulation (87.3% of dose at 30 min) in the liver at all times (except at 15 min) as compared with Glu-ST vesicles and PC liposomes, and maintained a high level unitl 8 h. The Glu-ST vesicles did not show significant differences from PC liposomes (except at 8 h). These results suggest that the high accumulation of the Gal-ST vesicles in the liver may be due to the galactose residue on the surface of the vesicles, and not to any property of AGV. It is still uncertain whether the galactose residue (50 mol%) on the surface of the vesicles is able to interact efficiently with the galactose binding receptor[3] on the surface of hepatocytes, or whether the vesicles (mean diameter 0.231 μm; Coulter Model N4) are able to pass unimpeded through the fenestration of the liver sinusoids, whose mean diameter is about 100 nm,[13] in order to interact with the hepatocytes. However, larger pores or gaps with a diameter of more than 0.25 μm in the endothelial cell lining have been reported,[14] so the results obtained in the present study suggest that the Gal-ST vesicles did bind to the hepatocytes through a specific interaction involving galactose. It is also possible that the vesicles bond to the Kupffer cells in liver sinusoids, because the presence of a similar receptor has been reported on the surface of the Kupffer cells.[15]

The spleen is also an RES-rich organ and traps large amounts of administered liposomes,[16] as does the liver. After the administration of PC liposomes, about 8—10% of the dose was found in the spleen, whereas 3—4% and about 5% were found after the

TABLE I. Tissue Distribution of Vesicles after Intravenous Injection in Mice

Tissue	Time (h)	Gal-ST vesicles	Glu-ST vesicles	PC liposomes
Blood	0.25	4.40±0.21[b]	6.03±3.49	8.87±1.90
	0.5	2.47±0.53	3.43±0.37[b]	1.59±0.70[a]
	1.0	1.33±0.20[b]	1.31±0.35[b]	0.56±0.15[a]
	4.0	0.43±0.03	0.70±0.18	0.49±0.16
	8.0	0.40±0.10	0.35±0.04	0.31±0.09
Liver	0.25	71.27±5.80	71.06±1.41	66.46±2.15
	0.5	87.33±4.47[a,b]	73.92±1.28	72.14±2.36
	1.0	82.72±2.13[a,b]	71.88±1.22	74.08±1.95
	4.0	79.93±2.20[a,b]	66.95±3.02	60.62±2.81
	8.0	74.60±1.94[a,b]	65.09±4.28[b]	51.69±2.14[a]
Spleen	0.25	3.41±0.52[a,b]	5.30±0.75[b]	7.96±0.88[a]
	0.5	3.93±1.03[b]	5.29±0.61[b]	7.87±1.13[a]
	1.0	3.20±0.19[a,b]	4.82±0.11[b]	9.59±0.79[a]
	4.0	3.51±0.08[b]	5.06±1.13[b]	9.86±1.12[a]
	8.0	3.34±0.32[a,b]	6.13±0.62	7.33±1.32
Lung	0.25	2.26±0.40[a,b]	0.84±0.15[b]	0.48±0.10[a]
	0.5	2.11±0.35[a,b]	0.62±0.06[b]	0.35±0.04[a]
	1.0	1.82±0.29[a,b]	0.33±0.05	0.24±0.06
	4.0	0.77±0.14[a,b]	0.27±0.02[b]	0.11±0.02[a]
	8.0	0.87±0.18[a,b]	0.31±0.04[b]	0.14±0.04[a]
Kidney	0.25	2.98±0.90[a,b]	1.12±0.06[b]	0.60±0.06[a]
	0.5	1.73±0.87	0.99±0.36	0.36±0.03
	1.0	1.11±0.19[a,b]	0.27±0.04	0.27±0.05
	4.0	0.64±0.06[a,b]	0.23±0.01	0.24±0.05
	8.0	0.70±0.11[a,b]	0.34±0.06	0.25±0.03

Values are means±S.D. of three mice, and are expressed as percent of the initial dose per whole tissue. *a*) Significant difference ($p<0.05$) against Glu-ST vesicles. *b*) Significant difference ($p<0.05$) against PC liposomes.

administration of Gal-ST vesicles and Glu-ST vesicles, respectively. These lower accumulations in the spleen might be due to the lower amount available to the spleen, because of the high accumulation in the liver. However, since low accumulation in the spleen was also observed in Glu-ST vesicles, low affinity to the spleen may be an intrinsic property of the AGV.

In the lung, higher accumulation of Gal-ST vesicles was observed as compared with both Glu-ST vesicles and PC liposomes. Glu-ST vesicles also showed higher accumulation in the lung than PC liposomes. The reasons for this phenomenon are not clear, but it seems unlikely that a size effect contributes to this accumulation, because these vesicles were sized by extrusion and dialysis as described above. Some effects of galactose residues and intrinsic properties of AGV probably contribute to the uptake of the vesicles into the lung. In contrast, the kidney showed an affinity for Gal-ST vesicles only. Therefore, this affinity seems to be due to the contribution of the galactose residues on the vesicle surface.

The present results suggest that the Gal-ST vesicles have a higher affinity for the liver, lung, and kidney and a lower affinity for the spleen than Glu-ST vesicles or PC liposomes. The Glu-ST vesicles have a higher affinity for the lung and lower affinity for the spleen. However, it seems that the affinity for the lung and kidney is not important with respect to the targeting of the vesicles, because the amounts accumulated in these tissues are a very small part of the initial dose.

Pharmacokinetic Simulation

In the previous section, we showed that the Gal-ST vesicles, Glu-ST vesicles, and PC liposomes have different tissue distribution profiles from each other after intravenous administration. However, this observation does not indicate how high affinity of Gal-ST vesicles for the liver is, or whether the low accumulation of the Gal-ST vesicles in the spleen is due to their low affinity for that tissue or to the small amount of the vesicles available to the spleen because of the high accumulation in the liver. Thus, we attempted to simulate the time courses of the tissue distribution in order to evaluate these phenomena quantitatively.

Simulation curves calculated with the parameter values listed in Table II and the mean values of experimental data after intravenous injection of PC liposomes are shown in Fig. 2. The calculated curves showed good agreement with the experimental data. In this calculation, the parameter values of the blood volume (V_1—V_6) and blood flow (Q_4—Q_6, and Q_t) were fixed as mentioned previously. The elimination or degradation process in the lung and kidney (which are not metabolic active tissues for these particles), and in the blood compartments of all the organs were neglected, because their contributions to the simulation result were assumed to be very low. The parameter values of the first-order rate constants from tissue to blood compartments were fixed as 0.1 h^{-1} in all the organs in order to simplify the calculation and to permit easy comparison of affinity among the tissue or types of vesicles. This was done

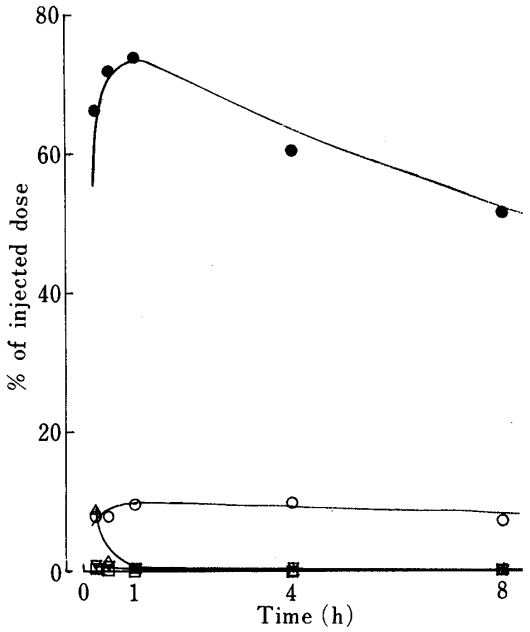

Fig. 2. Tissue Distribution Time Courses of PC Liposomes after Intravenous Injection in Mice

Points are mean values of three experiments each in the liver (●), spleen (○), blood (△), lung (□), and kidney (▽). The lines are calculated curves based on the model shown in Fig. 1 with the parameter values listed in Table II.

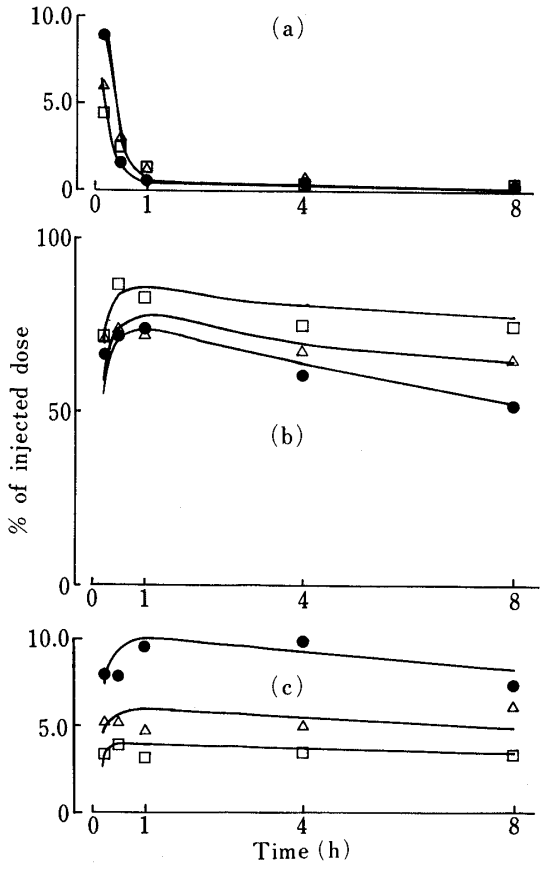

Fig. 3. Time Courses of Vesicles in Blood (a), Liver (b), and Spleen (c) after Intravenous Injection in Mice

Points are mean values of three experiments each after the administration of Gal-ST vesicles (□), Glu-ST vesicles (△), and PC liposomes (○). Lines are simulated curves calculated with the parameter values listed in Tables II and III.

TABLE II. Pharmacokinetic Parameters of PC Liposomes

V (ml)	Q (ml/h)	k (h^{-1})	k_e (h^{-1})
$V_1=1.40^{a)}$	$Q_4=105.20^{b)}$	$k_{37}=0.50^{c)}$	$k_e=0.60^{c)}$
$V_2=0.70^{a)}$	$Q_5=7.80^{a)}$	$k_{73}=0.10^{d)}$	
$V_3=0.04^{b)}$	$Q_6=76.50^{b)}$	$k_{48}=40.00^{c)}$	$k_{e_8}=0.04^{c)}$
$V_4=0.26^{b)}$	$Q_t=311.40^{b)}$	$k_{84}=0.01^{d)}$	
$V_5=0.02^{b)}$		$k_{59}=75.00^{c)}$	$k_{e_9}=0.02^{c)}$
$V_6=0.05^{b)}$		$k_{95}=0.01^{d)}$	
		$k_{610}=0.05^{c)}$	
		$k_{106}=0.10^{d)}$	

a) Parameter values obtained from the literature.[11] *b*) Parameter values obtained from the literature.[12] *c*) Parameter values to be determined by simulation. *d*) Fixed parameter values in simulation.

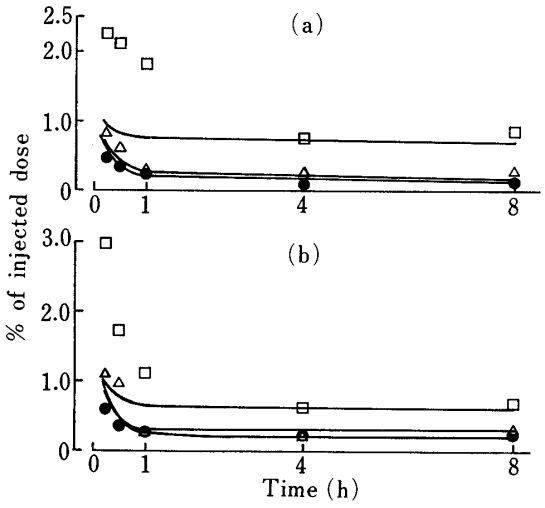

Fig. 4. Time Courses of Vesicles in Lung (a) and Kidney (b) after Intravenous Injection in Mice

All symbols are the same as those in Fig. 3.

TABLE III. Parameter Values Obtained by Simulation

	PC liposomes		Glu-ST vesicles		Gal-ST vesicles	
Tissue	k	k_e	k	k_e	k	k_e
Blood	—	0.60	—	0.60	—	0.50
Lung	0.50	—	0.70	—	3.00	—
Liver	40.00	0.04	40.00	0.03	60.00	0.015
Spleen	75.00	0.02	40.00	0.02	35.00	0.015
Kidney	0.50	—	0.70	—	2.00	—

k: rate constant for transfer of vesicles from blood to tissue. k_e: rate constant for elimination or degradation of vesicles.

because, in a preliminary simulation, it was considered that the values were not very different among the tissues and the simulation results were not much influenced by the exact values. Therefore, the simulation was carried out by changing the values of seven parameters which were those of transfer processes from blood to tissue compartments in the lung (k_{37}), liver (k_{48}), spleen (k_{59}), and kidney (k_{610}) and those of degradation or elimination processes in the blood (k_e), liver (k_{e_8}), and spleen (k_{e_9}). The parameter values of the first-order rate constants from blood to tissue in the organs correspond to the partition coefficients and reflect the affinity of the vesicles for the tissue, because the rate constant values from tissue to blood were

fixed at $0.1\,h^{-1}$ in all organs as mentioned above. Therefore, the affinities of the vesicles may be comparable in terms of these values. A set of parameter values (Table II) which showed a good agreement between the experimental data and calculated curves (Fig. 2) was found.

Based on the obtained values, the affinity of PC liposomes under physiological conditions is highest in the spleen (about twice that of the liver). The affinity for the lung or kidney was very low and the values were two orders of magnitude lower than that of the liver or spleen, and they were as low as five times that of release from tissue to blood. The process of degradation or elimination of PC liposomes in the blood was not rapid and it was also two orders of magnitude lower than that of uptake to the liver or spleen, whereas those in the liver and spleen were slower, being one more order of magnitude less than that in blood.

The simulations of the tissue distributions of Glu-ST vesicles and Gal-ST vesicles after injection were carried out in the same manner. The results are presented in Figs. 3 and 4. Parameter values used in these calculations are listed in Table III (only the changeable seven parameters mentioned above). The affinity of Glu-ST vesicles for the liver was not different from that of PC liposomes, whereas in the spleen, it was only about half of that of PC liposomes, being the same as that in the liver. The affinity values of the lung and kidney were slightly increased as compared with the PC liposomes. The degradation process in the liver was decreased and this explains the higher accumulation of Glu-ST vesicles in the liver as compared with PC liposomes at 8 h after injection (Table I).

On the other hand, in the case of the Gal-ST vesicles, the affinity for the liver was as high as 1.5 times that of PC liposomes or Glu-ST vesicles, whereas the degradation was slightly decreased. Therefore, the high accumulation of Gal-ST vesicles may be due to the high affinity of the vesicles for the liver, and not to high stability in the blood. In the spleen, the affinity value was as low as about a half that of PC liposomes and slightly lower than that of Glu-ST vesicles. This result suggests that the low accumulation of Gal-ST vesicles may be attributed mainly to the nature of the AGV and not to the sugar moiety on the surface of the vesicles. The parameter values of degradation in the liver and spleen were decreased, reflecting the higher accumulations in both tissues for a long time after administration. Though the calculation was carried out with higher parameter values of affinity to the lung and kidney (6 and 4 times those of PC liposomes, respectively), the calculated curves did not show a good fit to the experimental data, as shown in Fig. 4. This suggests that the accumulation in such a minor organ cannot be succssfully evaluated with the model presented here. The high affinity of the Gal-ST vesicles for the lung and kidney compared with PC liposomes, though the amount involved is a very small part of the initial dose, requires further study.

The rate constant values for degradation or elimination in the blood compartment (k_e) were $0.5—0.6\,h^{-1}$ and were similar for all types of vesicles, as shown in Table III, whereas the *in vitro* degradation rate constants of these vesicles were $0.2—0.3\,h^{-1}$, as obtained from the initial degradation phase of the vesicles incubated in rat plasma at 37 °C presented in the previous paper.[2] The discrepancy between the values obtained *in vivo* an *in vitro* suggested that these vesicles disintegrated more rapidly *in vivo* due to agitation of the blood flow and/or to the effects of other blood components.

Conclusion

It has become apparent in this study that AGV have a low affinity for the spleen and galactoside vesicles have a high affinity for the liver after intravenous administration in mice. Therefore, it is expected that the galactoside vesicles may be more effective drug carriers than PC liposomes if the target organ is the liver. The affinity for the tissues or the targeting efficiency of the vesicles after intravenous injection could be evaluated quantitatively by pharmacokinetic simulation based on the physiological flow model, and it is considered that this analysis may be a useful tool for quantitative evaluation of the affinity of drug carriers to

tissues. These results are important for the development of targetable drug carriers.

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