In Vivo Behavior of Liposomes Modified with a Novel Galactosyllipid Derivative

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We studied the *in vivo* behavior of galactosyllipid-modified liposomes for targeting the asialoglycoprotein receptor present on the surface of liver parenchymal cells. We examined the effects of the lipid composition of liposomes on the *in vivo* behavior of galactosyllipid-modified liposomes, and found good accumulation in the liver of liposomes of a high cholesterol content and liposomes made of lipids of a high gel-liquid crystalline phase transition temperature. The amount of modification with the galactosyllipid derivative required for effective targeting to the liver was found to be more than 5% of the total lipids. The concentration of galactosyllipid-modified liposomes was lower than that of control liposomes in all the organs except for the liver, showing high selectivity of galactosyllipid-modified liposomes for the liver. Hepatic accumulation of liposomes was inhibited by preinjection of asialofetuin. This result suggests that hepatic accumulation of {8-(2-hexadecyloctadecanoylamido)-3,6-dioxaoctyl}- β -D-galactoside (Gal-t-psa) liposome was involved with the asialoglycoprotein receptor in the liver. Therefore, it was concluded that our neogalactolipid-modified liposomes are useful for selective delivery to the liver.

Key words liposome; targeting; galactose; liver

As liposomes can encapsulate both hydrophilic and lipophilic drugs, and their *in vivo* behavior can be controlled by surface modification, liposomes have been examined extensively as carriers in a drug delivery system.^{2–4)}

Mechanisms by which glycoproteins with β -galactose residues are selectively recognized by the asialoglycoprotein receptors present on the surface of liver parenchymal cells and rapidly incorporated into the cells by endocytosis have been clarified.⁵⁾ With this knowledge, methods have been developed to make liposomes selectively accumulate in liver cells by modifying liposomes with synthetic glycolipids,⁶⁾ lactosylceramide,⁷⁾ asialofetuin⁸⁾ and fragments of the polysaccharide chain of asialofetuin.⁹⁾ However, there have been problems in the use of these compounds, such as their complicated structures, difficulty in industrial mass-production and their possible anti-genicity.

We synthesized various ligands for liposome modification with galactose as the homing device, evaluated the relationship between the glycolipid structure and liposome accumulation into the liver, and optimized the ligand structure for liposome modification. We previously reported that the triethyleneglycol spacer, and the branched lipid anchor, were appropriate for allowing ligands to modify liposomes.^{10,11)} Our neogalactolipid derivatives provide liver-directness on the liposomes. In addition, they have some advantages over the others, because they are chemically stable and their industrial mass-production is feasible. In this paper, we report the *in vivo* behaviors of galactosyllipid-modified liposomes under various conditions, considering their potential use in targeting the liver.

MATERIALS AND METHODS

Materials L- α -Phosphatidylcholine dipalmitoyl (DPPC), L- α -phosphatidylcholine dimyristoyl (DMPC), L- α -phosphatidylcholine distearoyl (DSPC), dicetyl phosphate

(DCP), cholesterol (CH), asialofetuin and inulin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). A phospholipid test kit was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Phosphate buffered saline (PBS) was from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). [³H]Inulin was from DuPont-NEN Research Products (Boston, MA, U.S.A.). All other chemicals were of reagent grade or better. Galactosyllipid, {8-(2-hexadecyloctadecanoylamido)-3,6-dioxaoctyl}- β -D-galactoside (Gal-t-psa; Fig. 1), was prepared as described previously.¹⁰⁾

Preparation of the Galactosyllipid Modified Liposomes Liposomes were prepared according to the method of Bangham *et al.*¹²⁾ with some modification. Galactosyllipid modified liposomes were prepared using 16 mM galactosyllipid together with 80 mM DPPC, 80 mM CH and 8 mM DCP. These lipids were dissolved in 10 ml of chloroform-methanol (1 : 1, v/v). The solvent was removed in a stream of nitrogen with heating, followed by drying under reduced pressure. The residues were hydrated with 8 ml of PBS containing cold 0.5% (w/v) inulin and 5.92 MBq [³H]inulin as an aqueous marker. The suspensions were vortexed mechanically and sonicated for 2 min with a bath type sonicator (Branson B1200, Emerson-Japan, Tokyo, Japan). The liposomes were extruded through polycarbonate membranes (Nuclepore Co., MA, U.S.A.) with pore sizes of 200, 100 and 80 nm in order, successively. These procedures were done at a temperature above the gel-liquid crystalline phase transition temperature (*T_c*) of the lipid materials. Non-encapsulated [³H]inulin was removed with an ultracentrifuge (Himac, Hitachi Koki Co., Ltd., Tokyo, Japan) at 60000 rpm for 15, 2 and 2 h in order,

Fig. 1. Structure of Galactosyllipid

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successively. The phospholipid concentration was measured by a phospholipid test kit and the phospholipid concentration of the liposomes was adjusted to 10 mmol/ml. The size of the liposomes was determined using a submicron particle analyzer (Nicom 370, Pacific Scientific, MD, U.S.A.). The mean diameter of liposomes ranged from 120–150 nm and did not change after the ultracentrifugation. The liposomes showed a multi-lamellar structure by negative-stain electron micrographs.

Animal Experiment Male Sprague-Dawley rats (180–230 g body weight) were given a single intravenous injection of 2.5 ml/kg of a liposome (25 μ mol phospholipid) suspension, containing about 1×10^6 dpm per dose, into the jugular vein under ether anesthesia. Blood samples were drawn from the contralateral jugular vein with heparinized syringes at the appropriate time point. The plasma was separated by centrifugation within 10 min. Immediately after the last sampling of blood, the rats were sacrificed under ether anesthesia and the heart, lung, thymus, pancreas, spleen, kidney, muscle, skin, brain, bone marrow and liver were removed and combusted using an automatic sample combustion system (ASC-113, Aloka, Tokyo, Japan). The ^3H was collected as $^3\text{H}_2\text{O}$. After adding a scintillator (Aquasol II, DuPont-NEN Research Products, Boston, MA, U.S.A.), the radioactivity was measured in a liquid scintillation counter (LSC-3600, Aloka, Tokyo, Japan).

RESULTS AND DISCUSSION

We evaluated the effects of the amount of cholesterol added, the type of phospholipids and Gal-t-psa modification amount on the *in vivo* behavior of galactosylipid-modified liposomes.

Effects of Cholesterol Content Figure 2 shows the elution profiles of the [^3H]inulin entrapped liposomes. Liposomes were incubated with rat plasma or PBS for 30 min, and subjected to gel filtration using a Sephacryl S-400.

As shown in Figs. 2d and 2e, gel filtration after the incubation of hard liposomes (DPPC:CH:DCP:Gal-t-psa = 10:10:1:2, 10:7.5:1:2) with the rat plasma resulted in the elution of [^3H]inulin into the void, as after the incubation with PBS. This result shows that the liposomes with this composition were not destroyed after incubation with rat plasma for 30 min. On the other hand, after the incubation of liposomes composed of DPPC:CH:DCP:Gal-t-psa = 10:5:1:2 with rat plasma, a part of [^3H]inulin eluted into the low molecular fraction, suggesting the destruction of some liposomes. After the incubation of liposomes composed of DPPC:CH:DCP:Gal-t-psa = 10:2.5:1:2, 10:0:1:2 with plasma, most of the [^3H]inulin was eluted into the low molecular weight fraction, suggesting the destruction of most liposomes. Therefore, when a drug is encapsulated in glycolipid

Fig. 2. Elution Profiles of Gal-t-psa Modified Liposomes on Sephacryl S-400 after Incubation with PBS Solution or Rat Plasma

Incubated solution (1 ml) was loaded to a Sephacryl S-400 column (1.2 $\times$ 15 cm) equilibrated with PBS (pH 7.4). (A), DPPC:CH:DCP:Gal-t-psa = 10:0:1:2; (B), DPPC:CH:DCP:Gal-t-psa = 10:2.5:1:2; (C), DPPC:CH:DCP:Gal-t-psa = 10:5.0:1:2; (D), DPPC:CH:DCP:Gal-t-psa = 10:7.5:1:2; (E), DPPC:CH:DCP:Gal-t-psa = 10:10:1:2; $\circ$, after incubation with PBS solution; $\bullet$, after incubation with rat plasma.

Fig. 3. Elimination of [^3H]Inulin Encapsulated Liposomes from Plasma after Intravenous Injection into the Jugular Vein in Rats

Each point represents the mean $\pm$ S.E. from three animals. $\circ$, DPPC:CH:DCP:Gal-t-psa = 10:1:1:2; $\bullet$, DPPC:CH:DCP:Gal-t-psa = 10:3:1:2; $\square$, DPPC:CH:DCP:Gal-t-psa = 10:5:1:2; $\blacksquare$, DPPC:CH:DCP:Gal-t-psa = 10:10:1:2.

Fig. 4. Tissue Distribution of [^3H]Inulin Encapsulated Liposomes after Intravenous Injection into the Jugular Vein in Rats (6h)

Each column represents the mean $\pm$ S.E. from three animals. $\square$, DPPC:CH:DCP:Gal-t-psa = 10:1:1:2; $\square$ (hatched), DPPC:CH:DCP:Gal-t-psa = 10:3:1:2; $\blacksquare$, DPPC:CH:DCP:Gal-t-psa = 10:5:1:2; $\blacksquare$ (dotted), DPPC:CH:DCP:Gal-t-psa = 10:10:1:2.

modified liposomes, a CH mole ratio of 5 or more is needed for stability in the plasma. These results were similar to our previous report.¹³⁾

The effects of cholesterol content on the plasma elimination and tissue distribution of liposomes are shown in Figs. 3 and 4, respectively. The rate of disappearance of liposomes in the plasma was lowest at DPPC:CH:DCP:Gal-t-psa = 10:5:1:2. The stability of liposomes in the plasma decreased as the mole fraction of cholesterol decreased below 5. The fast disappearance of liposomes at DPPC:CH:DCP:Gal-t-psa = 10:1:1:2 and 10:3:1:2 was considered to be due to the faster destruction of liposomes in the plasma. On the other hand, liposomes with a cholesterol content 10 (DPPC:CH:DCP:Gal-t-psa = 10:10:1:2) showed considerable accumulation in the liver, which would explain the fast disappearance of liposomes at this cholesterol content in plasma.

It has been reported that lactosylceramide-modified

Fig. 5. Elimination of [^3H]Inulin Encapsulated Liposomes from Plasma after Intravenous Injection into the Jugular Vein in Rats

Each point represents the mean $\pm$ S.E. from three animals. $\circ$, DMPC:CH:DCP:Gal-t-psa = 10:5:1:2; $\bullet$, DPPC:CH:DCP:Gal-t-psa = 10:5:1:2; $\square$, DPPC:CH:DCP:Gal-t-psa = 10:5:1:2; $\blacksquare$, DSPC:CH:DCP:Gal-t-psa = 10:5:1:2.

Fig. 6. Tissue Distribution of [^3H]Inulin Encapsulated Liposomes after Intravenous Injection into the Jugular Vein in Rats (6h)

Each column represents the mean $\pm$ S.E. from three animals. $\square$, DSPC:CH:DCP:Gal-t-psa = 10:5:1:2; $\square$ (hatched), DPPC:CH:DCP:Gal-t-psa = 10:5:1:2; $\blacksquare$, DPPC:CH:DCP:Gal-t-psa = 10:5:1:2; $\blacksquare$ (dotted), DMPC:CH:DCP:Gal-t-psa = 10:5:1:2.

liposomes with a high cholesterol content, "hard" liposomes, have shown a higher accumulation in the liver than ones with a lower cholesterol content.¹⁴⁾ It was suggested, therefore, that the higher concentration of liposomes with cholesterol contents of 5 (DPPC:CH:DCP:Gal-t-psa = 10:5:1:2) in plasma was due to their relatively high stability in plasma and to the low efficiency of their transportation to the liver. It was confirmed that "hard" liposomes with high cholesterol contents were efficient for liver targeting using Gal-t-psa modified liposomes.

Effects of Kinds of Phospholipids Effects of types of phospholipids constituting liposomes are shown in Figs. 5 and 6. Different kinds of liposomes were made of each of the following lipids, with different phase transition temperatures shown in parentheses: DMPC (T_c: 24 °C), DPPC (T_c: 41 °C) and DSPC (T_c: 54 °C).¹⁵⁾ DSPC liposomes showed the highest concentration in the plasma, and liposomes of a higher phase transition temperature

were found to stay longer in the plasma. Accumulation in the liver also paralleled the T_c , with DSPC liposomes showing the highest accumulation, although the difference was not large. In contrast, liposomes made of DMPC, which has the lowest T_c , were found to be transported to the kidney in the largest quantity. In the liver, bone marrow, spleen, lung, and heart, the concentration of liposomes within the organ was positively correlated with the T_c of the phospholipid constituting the liposomes, whereas in the kidney, the correlation was opposite. It was therefore concluded that stable liposomes consisting of a phospholipid with a higher phase transition temperature remain in the blood for a longer time and accumulate in various organs in a larger quantity. For liver targeting using Gal-t-psa modified liposomes, phospholipids showing a high T_c , such as DPPC or DSPC, proved to be better materials for making the liposomes.

Effects of Galactosylipid Modification Amounts The relationship between the extent of Gal-t-psa modification and the *in vivo* behaviors of liposomes following such modification was examined. Figure 7 shows changes in the plasma concentration of liposomes of varying Gal-t-psa modifications, and Fig. 8 shows their concentrations in various organs at 6 h after administration. As Gal-t-psa modification exceeded 5% in mole fractions (DPPC:CH:DCP:Gal-t-psa=10:10:1:1), the liposomes in the plasma clearly declined and accumulated in the liver. A significant difference was observed between DPPC:CH:DCP:Gal-t-psa=10:10:1:0.5 and DPPC:CH:DCP:Gal-t-psa=10:10:1:1 by Fisher's pairing *t*-test. However, no significant difference was observed between the control and DPPC:CH:DCP:Gal-t-psa=10:10:1:0.25, 10:10:1:0.5. So, a critical point exists between 0.5 and 1.0 of modified Gal-t-psa content. The liposome concentration in the liver leveled off at mole fractions above 10% (DPPC:CH:DCP:Gal-t-psa=10:10:1:2). It was therefore concluded that Gal-t-psa modification of more than 5% in mole fractions was needed.

Time Course of Gal-t-psa Modified Liposomes in Various Organs Tables I and II show changes in the concentrations of the control (DPPC:CH:DCP=10:10:1) and Gal-t-psa (DPPC:CH:DCP:Gal-t-psa=10:10:1:2) modified liposomes in the main organs over a 48 h period after injection. In the liver, Gal-t-psa modified liposomes were more abundant than the control liposomes at all the time points examined. The accumulation of

Fig. 7. Elimination of [3 H]Inulin Encapsulated Liposomes from Plasma after Intravenous Injection into the Jugular Vein in Rats

Each point represents the mean $\pm$ S.E. from three animals. $\circ$, DPPC:CH:DCP=10:10:1; $\bullet$, DPPC:CH:DCP:Gal-t-psa=10:10:1:0.25; $\square$, DPPC:CH:DCP:Gal-t-psa=10:10:1:0.5; $\blacksquare$, DPPC:CH:DCP:Gal-t-psa=10:10:1:1; $\triangle$, DPPC:CH:DCP:Gal-t-psa=10:10:1:2; $\blacktriangle$, DPPC:CH:DCP:Gal-t-psa=10:10:1:5.

Fig. 8. Tissue Distribution of [3 H]Inulin Encapsulated Liposomes after Intravenous Injection into the Jugular Vein in Rats (6h)

Each column represents the mean $\pm$ S.E. from three animals. $\square$, DPPC:CH:DCP=10:10:1; $\blacksquare$, DPPC:CH:DCP:Gal-t-psa=10:10:1:0.25; $\square$ with diagonal lines, DPPC:CH:DCP:Gal-t-psa=10:10:1:0.5; $\blacksquare$ with diagonal lines, DPPC:CH:DCP:Gal-t-psa=10:10:1:1; $\triangle$ with diagonal lines, DPPC:CH:DCP:Gal-t-psa=10:10:1:2; $\blacktriangle$ with diagonal lines, DPPC:CH:DCP:Gal-t-psa=10:10:1:5.

Table 1. Tissue Concentration of Control Liposome (DPPC:CH:DCP=10:10:1)

Organs	% of dose/g tissue							
	5 min	30 min	1 h	2 h	6 h	12 h	24 h	48 h
Blood	5.9059 $\pm$ 0.1960	6.2928 $\pm$ 0.0568	4.3986 $\pm$ 0.1903	5.3373 $\pm$ 0.2179	2.8263 $\pm$ 0.0944	2.0866 $\pm$ 0.1883	1.0650 $\pm$ 0.0491	0.0290 $\pm$ 0.0148
Heart	0.4580 $\pm$ 0.0063	0.6436 $\pm$ 0.0174	0.4522 $\pm$ 0.0259	0.5095 $\pm$ 0.0065	0.2477 $\pm$ 0.0091	0.3205 $\pm$ 0.0144	0.3034 $\pm$ 0.0229	0.1372 $\pm$ 0.0103
Lung	1.3358 $\pm$ 0.1024	1.0568 $\pm$ 0.0909	0.8263 $\pm$ 0.1435	0.8808 $\pm$ 0.0249	0.5255 $\pm$ 0.0439	0.7741 $\pm$ 0.0280	0.9334 $\pm$ 0.0777	0.6102 $\pm$ 0.0782
Spleen	2.6108 $\pm$ 0.2398	7.6396 $\pm$ 1.2558	8.8255 $\pm$ 0.0184	13.3793 $\pm$ 0.5604	26.1028 $\pm$ 3.5199	23.3324 $\pm$ 1.2118	27.7640 $\pm$ 1.2922	24.6514 $\pm$ 2.7420
Kidney	0.9374 $\pm$ 0.1165	0.8318 $\pm$ 0.0525	0.5096 $\pm$ 0.0415	0.5859 $\pm$ 0.0365	0.5404 $\pm$ 0.0306	0.5112 $\pm$ 0.0311	0.5306 $\pm$ 0.0340	0.4077 $\pm$ 0.0119
Muscle	0.0937 $\pm$ 0.0113	0.0651 $\pm$ 0.0078	0.0683 $\pm$ 0.0137	0.0806 $\pm$ 0.0148	0.0232 $\pm$ 0.0074	0.0701 $\pm$ 0.0010	0.0691 $\pm$ 0.0208	0.0196 $\pm$ 0.0015
Bone marrow	0.9390 $\pm$ 0.0385	1.3700 $\pm$ 0.0657	1.2193 $\pm$ 0.1143	2.0637 $\pm$ 0.2895	2.8775 $\pm$ 0.2932	2.6988 $\pm$ 0.1555	3.0283 $\pm$ 0.2267	2.7652 $\pm$ 0.3824
Liver	0.5626 $\pm$ 0.0176	0.5626 $\pm$ 0.0176	0.7670 $\pm$ 0.0417	1.0953 $\pm$ 0.0272	1.2276 $\pm$ 0.1016	1.6001 $\pm$ 0.1767	2.2344 $\pm$ 0.2573	2.9753 $\pm$ 0.2425
Plasma	10.9303 $\pm$ 0.3531	11.2961 $\pm$ 0.1632	8.7700 $\pm$ 0.3678	9.5345 $\pm$ 0.2888	4.9076 $\pm$ 0.1677	3.7150 $\pm$ 0.3207	2.1171 $\pm$ 0.1554	0.0765 $\pm$ 0.0306

Each point represents the mean $\pm$ S.E. from three animals.

Table 2. Tissue Concentration of Gal-t-psa Liposome (DPPC:CH:DCP:Gal-t-psa = 10:10:1:2)

Organs	% of dose/g tissue							
	5 min	30 min	1 h	2 h	6 h	12 h	24 h	48 h
Blood	3.6744 ± 0.3057	2.1106 ± 0.1927	1.0772 ± 0.1903	0.8622 ± 0.1308	0.2541 ± 0.0126	0.3186 ± 0.1061	0.1322 ± 0.0341	0.0115 ± 0.0025
Heart	0.3866 ± 0.0282	0.2357 ± 0.0238	0.1279 ± 0.0259	0.0834 ± 0.0165	0.0267 ± 0.0012	0.0607 ± 0.0211	0.0571 ± 0.0129	0.0279 ± 0.0049
Lung	3.8641 ± 2.4234	0.6666 ± 0.0614	0.7702 ± 0.1435	0.3250 ± 0.0426	0.1902 ± 0.0302	0.2184 ± 0.0442	0.1802 ± 0.0118	0.1243 ± 0.0332
Spleen	3.5013 ± 0.6053	5.0973 ± 0.1265	4.0714 ± 0.0184	5.9852 ± 0.3978	3.6997 ± 0.4340	5.0306 ± 0.7575	4.3557 ± 0.0805	3.5137 ± 0.5018
Kidney	3.8572 ± 1.4309	1.1751 ± 0.0321	0.9884 ± 0.0415	0.2453 ± 0.0192	0.5384 ± 0.1967	0.3507 ± 0.0394	0.2680 ± 0.0270	0.2868 ± 0.0215
Muscle	0.0934 ± 0.0085	0.0575 ± 0.0098	0.0194 ± 0.0137	0.0267 ± 0.0071	0.0075 ± 0.0014	0.0321 ± 0.0025	0.0232 ± 0.0038	0.0081 ± 0.0037
Bone marrow	0.8422 ± 0.0966	1.0284 ± 0.1323	0.7086 ± 0.1143	0.9345 ± 0.0500	0.5845 ± 0.0327	0.6492 ± 0.1880	0.9683 ± 0.0506	0.7697 ± 0.2278
Liver	2.3997 ± 0.2708	4.2915 ± 0.2454	4.5270 ± 0.0417	5.7962 ± 0.7060	5.7590 ± 0.3392	5.7386 ± 0.0277	4.1702 ± 0.3509	3.6849 ± 0.1214
Plasma	6.3003 ± 0.5697	4.1456 ± 0.3713	2.0173 ± 0.3678	1.4928 ± 0.2154	0.4296 ± 0.0364	0.6184 ± 0.1839	0.2488 ± 0.0679	0.0078 ± 0.0007

Each point represents the mean ± S.E. from three animals.

Fig. 9. Elimination of [^3H]Inulin Encapsulated Liposomes from Plasma after Intravenous Injection into the Jugular Vein in Rats

Each point represents the mean ± S.E. from three animals. ○, Control liposome (DPPC:CH:DCP=10:10:1); ●, Control liposome + preinjection of asialofetuin (25 mg/kg); □, Gal-t-psa liposome (DPPC:CH:DCP:Gal-t-psa=10:10:1:2); ■, Gal-t-psa liposome + preinjection of asialofetuin (25 mg/kg).

Gal-t-psa modified liposomes in the liver peaked at 1 h after administration, while the concentration of the control liposomes gradually increased up to 12 h, presumably because the control liposomes existing in the plasma were continuously taken up by the liver. In the heart, lung, spleen, muscle, and bone marrow, on the other hand, the control liposomes existed at a higher concentration than Gal-t-psa modified liposomes at all time points except immediately after administration. This indicates that Gal-t-psa modified liposomes were selectively transported to the liver.

Effects of Asialofetuin Preadministration on the *in Vivo* Behavior of Liposomes Since it was not clear whether the selective delivery of Gal-t-psa modified liposomes to the liver was mediated by its interaction with the asialoglycoprotein receptor, asialofetuin (25 mg/kg) was injected intravenously 5 min before the administration of liposomes, and the concentrations of the control (DPPC:CH:DCP=10:10:1) and Gal-t-psa (DPPC:CH:DCP:Gal-t-psa=10:10:1:2) modified liposomes in the plasma were measured. Figure 9 shows the changes in the liposome concentrations in the plasma up to 30 min after administration of the liposomes, and Fig. 10 shows the

Fig. 10. Hepatic Accumulation of [^3H]Inulin Encapsulated Liposomes after Intravenous Injection into the Jugular Vein in Rats (30 min)

Control: DPPC:CH:DCP=10:10:1, Gal-t-psa: DPPC:CH:DCP:Gal-t-psa=10:10:1:2. Each column represents the mean ± S.E. from three animals.

liposome concentrations in the liver at 30 min after administration. It can be seen that the change with time in the concentrations of Gal-t-psa modified liposomes in the plasma became almost the same as that of the control in the presence of asialofetuin. Its concentration in the liver was also significantly reduced by the preadministration of asialofetuin, though it was still slightly higher than the control. These results strongly suggest the involvement of an asialoglycoprotein receptor in the liver in the accumulation of Gal-t-psa modified liposomes in the liver.

CONCLUSIONS

The following conditions were found from the results described above to be conducive to efficient liver targeting using Gal-t-psa modified liposomes: liposomes with a high cholesterol content and high Tc phospholipid level, and modified with Gal-t-psa to 5% or more in a mole fraction, are more efficiently transported to the liver. Furthermore, the amount of Gal-t-psa modified liposomes distributed in other organs was relatively small, indicating its suitability for selective delivery to the liver. Since the compounds we used for lipid modification can be mass-produced industrially, their use in the future of Gal-t-psa modified liposomes for the selective delivery

of drugs to the liver seems promising.

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