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Asialoglycoprotein Receptor-Mediated Gene Transfer Using Novel Galactosylated Cationic Liposomes

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We synthesized three novel galactosylated cholesterol derivatives, cholesten-5-yloxy-N-(4-((1-imino-c-β-D-thiogalactosyl-ethyl)amino)butyl)formamide (Gal-C4-Chol) and its ethyl formamide and hexyl formamide analogues (Gal-C2-Chol, Gal-C6-Chol), to prepare liposomal gene carriers possessing the cationic charge necessary for plasmid DNA binding and galactose residues as a targetable ligand for liver parenchymal cells. Liposome/DNA complexes prepared with these lipids showed low cytotoxicity in human hepatoma HepG2 cells. Gal-C4-Chol/DC-Chol/DOPE(3:3:4) liposomes, consisting of 3:3:4 mixtures of Gal-C4-Chol, 3β[N′,N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol), and dioleoylphosphatidylethanolamine (DOPE), showed higher transfection activity and [^{32}P]DNA uptake than DC-Chol/DOPE(6:4) liposomes. The presence of 20 mM galactose significantly inhibited both transfection efficiency and uptake of DNA of Gal-C4-Chol/DC-Chol/DOPE(3:3:4) and Gal-C4-Chol/DOPE(6:4) liposomes, but not those of DC-Chol/DOPE(6:4) liposomes. These results indicate that the liposome/DNA complexes prepared using novel galactosylated cholesterol derivatives are efficiently recognized by asialoglycoprotein receptors and internalized and lead to gene expression. In addition, we found that the galactosylated cholesterol derivative with a longer spacer showed higher transfection activity. © 1998 Academic Press

Gene transfer to hepatocytes should be of great therapeutic potential since hepatocytes are responsible for the synthesis of a wide variety of proteins, which play important physiological roles in or outside the hepatocytes after various post-translational modifications and translocation/secretion. There has been much interest in in vivo gene transfer to the liver, as an alternate to ex vivo methods, which require invasive surgery (1). However, most of the established viral and non-viral gene carriers lack cell specificity and, therefore, their in vivo application is limited.

Hepatocytes exclusively express large numbers of high affinity cell-surface receptors that can bind asialoglycoproteins and subsequently internalize them to the cell interior. In late 1980s, Wu et al. demonstrated successful in vivo gene transfer to hepatocytes, using poly-L-lysine linked with asialoorosomucoid (2,3). In addition, Hara et al. showed that asialofetuin-labeled liposome encapsulating plasmid DNA is effective in gene expression (4-6). However, introduction of asialoglycoproteins to the DNA carriers is complicated, so that the carriers might have some problems in reproducibility etc. We have established the methods of introducing sugar moieties directly to various molecular species and developed various macromolecular drug carrier systems and protein derivatives which show superior liver targeting via receptor mediated endocytosis (7-11). Our method would be much better for the development of liver-specific DNA carrier. On the other hand, it is also important to prevent DNA from degrading in the endosome/lysosomes after being internalized. It has been known that the pH-sensitive dioleoylphosphatidylethanolamine (DOPE) enhances cationic liposome-mediated gene transfer by accelerating endosomal escape of DNA (12,13). Taken together with these points, efficient receptor-mediated gene transfer to hepatocytes could be achieved if galactose moieties are directly introduced into the cationic lipid/DOPE system.

In the present study, we synthesized novel galactosylated cholesterol derivatives, cholesten-5-yloxy-N-(4-((1-imino-c-β-D-thiogalactosyl-ethyl)amino)alkylformamide, for gene delivery to hepatocytes. These derivatives possess bi-functional properties i.e. an imino group for binding to plasmid DNA via electrostatic interaction and a galactose residue for the cell surface

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receptors in hepatocytes. Unlike neutral molecules, addition of such a cationic group dose not reduce the charge density of the cationic liposomes. Therefore, a high density of galactose residues can be provided on the liposome surface without affecting the binding ability of cationic liposomes to DNA in these galactosylated cholesterol derivatives. In the present study, the gene expression and uptake of plasmid DNA complexed with cationic liposomes containing the galactosylated lipids were investigated in human hepatoma HepG2 cells which are known to express asialoglycoprotein receptors. Their feasibilities of using such derivatives was compared with that of 3β[N′,N′,N′-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol) liposome which are known to have a very high potential for in vitro gene transfection (14-17).

EXPERIMENTAL METHODS

Materials. N-(4-aminoethyl) carbamic acid tert-butyl ester, N-(4-aminobutyl) carbamic acid tert-butyl ester, and N-(4-aminohexyl) carbamic acid tert-butyl ester were obtained from Tokyo Chemical Industry (Tokyo, Japan). [α-^{32}P]-dCTP (3000 Ci/mmol) was obtained from Amersham (Tokyo, Japan). Cholesteryl chloroformate and dioleoylphosphatidylethanolamine (DOPE) were obtained from Sigma Chemicals (St. Louis, MO) and Avanti Polar-Lipids (Alabaster, AL), respectively. 3β[N′,N′,N′-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol) was synthesized according to the published method (14). Dulbecco's modified Eagle's minimum essential medium (DMEM) and Hank's buffered salt solution (HBSS) were obtained from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum (FBS) was obtained from Biowhittaker (Walkersville, MD). Opti-MEM I and other culture reagents were obtained from Gibco BRL (Grand Island, NY). All other chemicals were of the highest purity available.

Construction and preparation of plasmid DNA (pCMV-Luc). pCMV-Luc was constructed by subcloning the Hind III/Xba I firefly luciferase cDNA fragment from pGL3-control vector (Promega, Madison, WI) into the polylinker of pcDNA3 vector (Invitrogen, Carlsbad, CA). Plasmid DNA was amplified in the E. coli strain DH5α, isolated, and purified using a QIAGEN Plasmid Giga Kit (QIAGEN GmbH, Hilden, Germany). Purity was confirmed by 1 % agarose gel electrophoresis followed by ethidium bromide staining and the DNA concentration was measured by UV absorption at 260 nm. The plasmid was labeled with [α-^{32}P]-dCTP by nick translation (18).

Synthesis of cholesten-5-yloxy-N-(4-((1-imino-c-β-D-thiogalactosyl-ethyl)amino)butyl)formamide (Gal-C4-Chol). Cholesteryl chloroformate (1 mol) and N-(4-aminobutyl) carbamic acid tert-butyl ester (1.1 mol) were reacted in 20 ml chloroform for 24 h at room temperature. Five milliliters trifluoroacetic acid was added and the reaction continued for 4 h at 4°C. After the solvents were evaporated in vacuo, 10 ml hexane was added to the resultant syrup and the N-(4-aminobutyl)-(cholesten-5-yloxyl)formamide preparation was obtained.

2-Imino-2-methoxyethyl-1-thiogalactoside (IME-thiogalactoside) was prepared as reported previously (8). Briefly, cyanomethyl-1-thiogalactoside (5 mol) was reacted with 20 ml 0.01 M sodium methoxide methanolic solution at room temperature for 24 h. The solvent was evaporated in vacuo and the resultant syrup was dissolved in 20 ml pyridine containing 1.1 mol triethylamine.

N-(4-aminobutyl)-(cholesten-5-yloxyl)formamide was added to the IME-thiogalactoside solution and reacted for 24 h at room temperature. After the reaction mixture was evaporated in vacuo, the resultant material was suspended in water, and dialyzed against distilled water for 48 h using a dialysis membrane (12 kDa cut-off). After the dialyzate was lyophilized, the crude product was purified by recrystalization three times in ethylacetate. ^{1}H-NMR (200MHz, $CDCl_3$): Gal-C4-Chol : 0.5-2 (m, cholesteryl skeleton), 2.3 (m, N-CH_2), 2.9 (broad s, N-CH_2), 3.1 (broad s, S-CH_2), 5.4 (s, C-NH). FAB-MS m/z: 736 (M+).

Cholesten-5-yloxy-N-(4-((1-imino-c-β-D-thiogalactosyl-ethyl)amino)-ethyl)-formamide (Gal-C2-Chol) and cholesten-5-yloxy-N-(4-((1-imino-c-β-D-thiogalactosyl-ethyl)amino)hexyl)formamide (Gal-C6-Chol) were synthesized in a similar manner, using N-(4-aminoethyl) carbamic acid tert-butyl ester and N-(4-aminohexyl) carbamic acid tert-butyl ester, respectively. Gal-C2-Chol: ^{1}H-NMR (200MHz, $CDCl_3$) 0.5-1.8 (m, cholesteryl skeleton), 2.0 (m, N-CH_2), 2.6 (broad s, N-CH_2), 3.0 (broad s, S-CH_2), 5.5 (s, C-NH). FAB-MS m/z: 709 (M+). Gal-C6-Chol: ^{1}H-NMR (200MHz, $CDCl_3$) 0.5-2.0 (m, cholesteryl skeleton), 2.2 (m, N-CH_2), 2.7 (broad s, N-CH_2), 3.2 (broad s, S-CH_2), 5.5 (s, C-NH). FAB-MS m/z: 765 (M+).

Preparation and characterization of cationic liposomes. Cholesterol derivatives of 1.2 μmol and 0.8 μmol DOPE were dissolved in chloroform, vacuum-desiccated, and resuspended in 1 ml sterile 20 mM HEPES buffer (pH 7.8). The suspension was sonicated for 10 min in a bath sonicator and passed through a polycarbonate membrane filter (pore size of 0.45 μm).

The particle size of the liposomes was measured in a dynamic light scattering spectrophotometer (LS-900, Otsuka Electronics, Osaka, Japan). The zeta potential of the liposomes was determined with a laser electrophoresis zeta-potential analyzer (LEZA-500T, Otsuka Electronics).

Transfection experiment. HepG2 cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in DMEM supplement with 10 % FBS at 37°C under an atmosphere of 5 % CO_2 in air.

The cells were plated on a 6-well cluster dish at a density of 2×10^5 cells/10.5 cm^2 and cultivated in 2 ml DMEM supplemented with 10 % FBS. After 24 h, the culture medium was replaced with Opti-MEM I containing 0.5 μg/ml plasmid DNA and cationic liposomes. Six hours later, the incubation medium was replaced again with DMEM supplement with 10 % FBS and incubated for an additional 42 h. Then, the cells were scraped and suspended in 200 μl pH 7.4 phosphate-buffered saline (PBS). One hundred microliters cell suspension was subjected to three cycles of freezing (liquid N_2 for 3 min) and thawing (37°C for 3 min), followed by centrifugation at 10,000 g for 3 min. The supernatants were stored at −20°C until the luciferase assays were performed. Ten microliters supernatant was mixed with 100 μl luciferase assay buffer (Picagene, Toyo Ink, Tokyo, Japan) and the light produced was immediately measured using a luminometer (Lumat LB 9507, EG & G Berthold, Bad Wildbad, Germany). The activity is indicated as the relative light units per mg protein. The protein content of the cell suspension in PBS was determined by a modified Lowry method using BSA as a standard (19). The effect of the copresence of galactose and mannose was determined in the same system. We also tested NIH 3T3 cell in a similar method.

Uptake experiment. The HepG2 cells were plated on a 12-well cluster dish at a density of 7.5×10^4 cells/3.8 cm^2 and cultivated in 800 μl DMEM supplement with 10 % FBS. Twenty-four hours later, the culture medium was replaced with an equivalent volume of HBSS containing 1 kBq/ml [^{32}P]DNA, 0.5 μg/ml cold DNA and cationic liposomes (2.5 μg/ml DC-Chol liposome or 5 μg/ml galactosylated liposomes). After incubation for given time periods, the solution was immediately removed by aspiration, the cells were washed five times with ice-cold HBSS buffer and solubilized in 1 ml 0.3 N NaOH solution with 10 % Triton X-100. The radioactivity was measured by liquid scintillation counting (LSC-500, Beckman, Tokyo, Japan) and the protein content was determined by modified the Lowry method (19). The effect of copresence of galactose and mannose was determined in the same system.

TABLE 1

Particle Size and Zeta Potential of Various Cationic Liposomes in pH 7.8 HEPES Buffer

Liposome	Composition (mol) Gal-C4-Chol:DC-Chol:DOPE	Particle size (nm)	Zeta potential (mV)
DC-Chol/DOPE(6:4)	0:6:4	210.3 ± 21.8	39.8 ± 2.9
Gal-C4-Chol/DOPE(6:4)	6:0:4	195.6 ± 15.2	34.5 ± 4.6
Gal-C4-Chol/DC-Chol/DOPE(3:3:4)	3:3:4	211.0 ± 13.2	37.1 ± 3.5

Note. Values are means ± SD (n=3).

Cytotoxicity test. Cytotoxicity was evaluated by MTT assay. The cells plated on a 96-well plate at a density of 5.6×10^4 cells/0.28cm^2 were incubated with liposome/DNA complexes in 100 μl OptiMEM I for 6 h. The cells were incubated for an additional 4 h, after addition of 0.5 mg/ml MTT solution and lysed overnight at 37°C with 10 % SDS solution. The absorbance was measured at test and reference wavelengths of 570 and 660 nm, respectively, in a two-wavelength microplate photometer (Bio-Rad Model 450, Hercules, CA).

RESULTS

Three types of liposome were prepared with various ratios of DC-Chol, Gal-C4-Chol, and DOPE (6:0:4, 0:6:4, and 3:3:4). The prepared liposomes are almost similar in particle size and zeta potential as summarized in Table 1. Plasmid DNA encoding a luciferase gene was complexed with these cationic liposomes at various ratios (1:2.5 ~ 1:15(w/w)). MTT assay showed all complexes possessed low cytotoxicities in HepG2 cells (data not shown).

Figure 1 shows the expression of luciferase gene in HepG2 cells treated with the DNA/cationic liposome complexes. Three cationic liposomes showed different optimal DNA/liposome ratios for gene expression. The greatest gene expression with DC-Chol/DOPE(6:4) liposome was observed at a DNA/liposome ratio of 1:5, whereas that for Gal-C4-Chol/DC-Chol/DOPE(3:3:4) and Gal-C4-Chol/DOPE(6:4) liposomes was shown at a ratio of 1:10. When gene expression was compared at their optimal ratios, Gal-C4-Chol/DC-Chol/DOPE(3:3:4) liposomes were the most effective. The maximum transfection activities of DC-Chol/DOPE(6:4) liposomes and Gal-C4-Chol/DOPE(6:4) liposomes were almost the same and half that of Gal-C4-Chol/DC-Chol/DOPE(3:3:4) liposomes. As shown in Fig. 2(A), the presence of 20 mM galactose significantly inhibited the gene expression of Gal-C4-Chol/DC-Chol/DOPE(3:3:4) and Gal-C4-Chol/DOPE(6:4) liposomes, but not that of DC-Chol/DOPE(6:4) liposomes. On the other hand, gene expression with all DNA/liposome complexes was not inhibited by 20 mM mannose (Fig.2(B)). These results suggest that DNA complexed with galactosylated liposomes might be recognized by the asialoglycoprotein receptors on HepG2 cells.

Gene expression by the DNA/liposome complexes was also investigated in NIH 3T3 cells for comparison (Fig. 3). All the DNA/liposome complexes gave higher gene expression at a DNA/liposome ratio of 1:10.

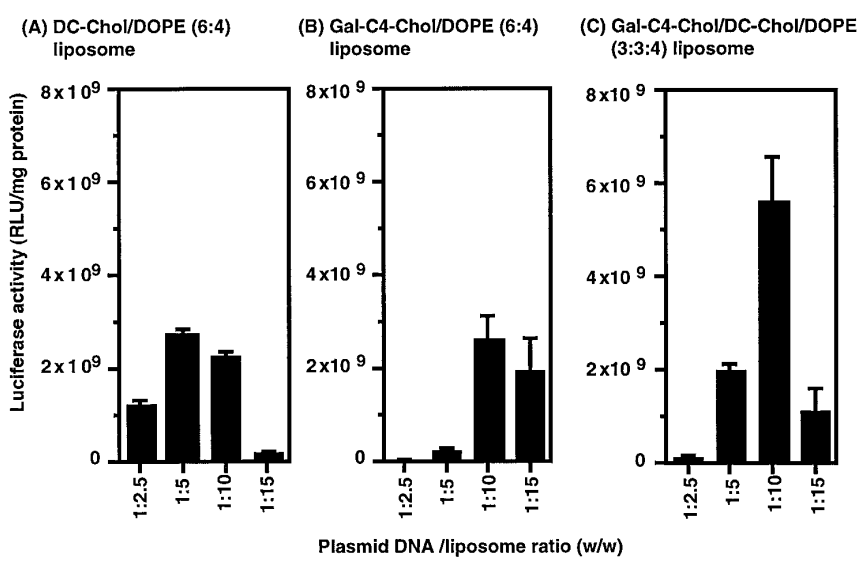

FIG. 1. Transfection activity of DNA/liposome complexes at various ratios (w/w) in HepG2 cells. DNA concentration was fixed at 0.5 μg/ml in all experiments. Each value represents the mean ± S.D. values (n=3).

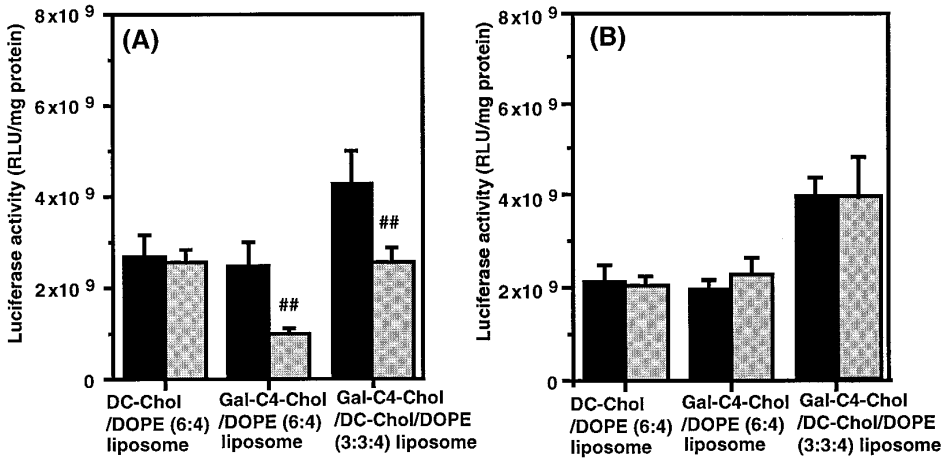

FIG. 2. Effect of copresence of 20 mM galactose (A) and mannose (B) on the transfection activity of DNA/liposome complexes at their optimal liposome/DNA ratios in HepG2 cells. Cells were transfected with DNA/liposome complexes in the presence (▨) and absence (■) of either galactose or mannose. DNA concentration was fixed at 0.5 μg/ml in all experiments. DNA/liposome ratios are 1:5 (w/w): DC-Chol/DOPE(6:4), 1:10 (w/w): Gal-C4-Chol/DOPE(6:4), 1;10 (w/w): Gal-C4-Chol/DC-Chol/DOPE(3:3:4), respectively. Each value represents the mean ± S.D. values (n=3). Stastical analysis was performed by analysis of variance ($^{\#\#}P<0.01$).

Transfection activity of Gal-C4-Chol/DOPE(6:4) and Gal-C4-Chol/DC-Chol/DOPE(3:3:4) liposomes at this ratio was significantly lower than that of DC-Chol/DOPE(6:4) liposomes. Unlike the case of HepG2 cells, the presence of 20 mM galactose did not inhibit the gene expression.

In the galactosylated cholesterol derivatives, the effect of the spacer length between the anchor and galactose residues was examined. Figure 4 shows the expression of luciferase in HepG2 transfected by pCMV-Luc with either Gal-C2-Chol/DC-Chol/DOPE(3:3:4), Gal-C4-Chol/DC-Chol/DOPE(3:3:4), or Gal-C6-Chol/DC-Chol/DOPE(3:3:4) liposomes. As the spacer of the galactosylated lipid became longer, the transfection activity of the DNA/liposome complexes became higher. In addition, the presence of 20 mM galactose reduced the expression of gene to the same level in all the galactosylated liposome/DNA complexes.

The cellular uptake time-courses of [^{32}P]DNA complexed with the liposomes was also examined in HepG2 cells (Fig. 5). The highest uptake of [^{32}P]DNA was observed when it was complexed with Gal-C4-Chol/DC-Chol/DOPE(3:3:4) liposomes. The uptake of [^{32}P]DNA complexed with Gal-C4-Chol/DOPE(6:4) liposomes was comparable with that with DC-Chol/DOPE (6:4) lipo-

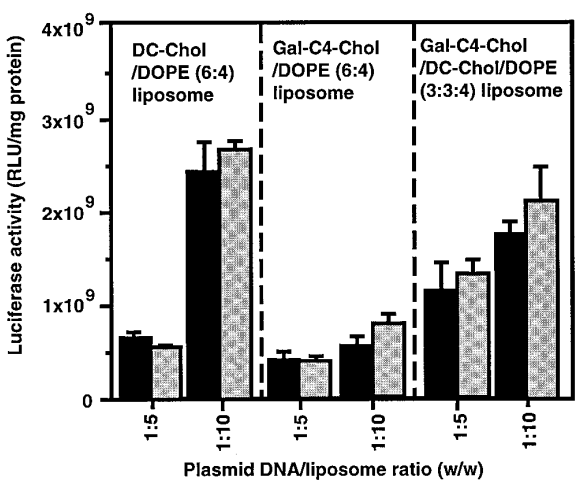

FIG. 3. Transfection activity of DNA/liposome complexes in NIH 3T3 cells. DNA (0.5 μg/ml) was complexed with cationic liposomes at the ratios of 1:5 and 1:10. Cells were transfected with DNA/liposome complexes in the presence (▨) and absence (■) of 20 mM galactose. Each value represents the mean ± S.D. values (n=3).

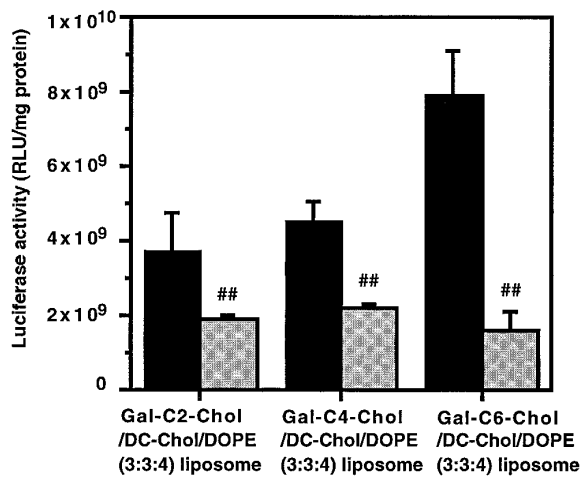

FIG. 4. Effect of copresence of 20 mM galactose on transfection activity of DNA/liposome complexes with different spacer lengths in HepG2 cells. Cells were transfected with DNA/liposome complexes in the presence (▨) and absence (■) of 20 mM galactose. DNA (5 μg/ml) was used at a ratio of 1:10 (DNA:liposome, w/w) in all experiments. Each value represents the mean ± S.D. values (n=3). Stastical analysis was performed by analysis of variance ($^{\#\#}P<0.01$).

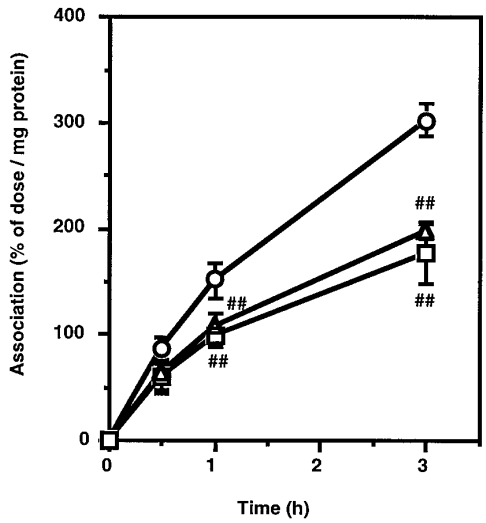

FIG. 5. Cellular association time-courses of ^{32}P-labeled DNA complexed with DC-Chol/DOPE(6:4) (□), Gal-C4-Chol/DOPE(6:4) (△), and Gal-C4-Chol/DC-Chol/DOPE(3:3:4) (○) liposomes in HepG2 cells at 37°C. DNA concentration was fixed at 0.5 μg/ml in all experiments. DNA/liposome ratios are 1:5 (w/w): DC-Chol/DOPE(6:4), 1:10 (w/w): Gal-C4-Chol/DOPE(6:4), 1:10 (w/w): Gal-C4-Chol/DC-Chol/DOPE(3:3:4), respectively. Each value represents the mean ± S.D. values (n=3). Stastical analysis was performed by analysis of variance ($^{\#\#}P<0.01$).

somes. In the presence of 20 mM galactose, the uptake of [^{32}P]DNA with Gal-C4-Chol/DC-Chol/DOPE(3:3:4) and Gal-C4-Chol/DOPE(6:4) liposomes up to 3 h was significantly reduced, but this was not the case with DC-Chol/DOPE(6:4) (Fig. 6). These results correspond to those of the transfection experiments.

DISCUSSION

In the present study, we developed novel galactosylated lipids with bifunctional properties of plasmid DNA binding via electrostatic interaction and a high affinity for hepatocytes via their asialoglycoprotein receptors. These properties of the galactosylated lipids were confirmed by the fact that liposomes with galactosylated lipids and a neutral lipid DOPE were cationic (Table 1) and the gene expression and uptake in HepG2 cells of plasmid DNA complexed with the galactosylated liposomes were inhibited by excess free galactose (Fig.2(A) and Fig.6) but not by free mannose (Fig. 2(B)). In NIH 3T3 cells lacking asialoglycoprotein receptors, the galactosylated liposomes showed less transfection activities than DC-Chol/DOPE(6:4) liposomes and the activities were not inhibited by free galactose (Fig. 3).

The transfection activity of Gal-C4-Chol/DC-Chol/DOPE(3:3:4) liposomes was the highest followed by Gal-C4-Chol/DOPE(6:4) and DC-Chol/DOPE(6:4) liposomes. The optimal ratio of the two types of galactosylated liposome/DNA complexes was higher than that of the DC-Chol/DOPE(6:4) liposome/DNA complex. Yang and Huang (16) indicated that aggregation of DC-Chol liposome/DNA complexes occurs at high ratios of liposome to DNA, hampering their transfection activity. In the case of galactosylated liposomes, the hydrophilic galactose residues sticking out of the liposome surface seem to prevent the complex from aggregating. In fact, the turbidity of the galactosylated liposome/DNA complexes was lower than that of the DC-Chol/DOPE(6:4) liposome/DNA complex. This might explain the high optimal ratios of the galactosylated liposome/DNA complex.

It is important to provide a high density of galactose residues on the liposome surface for effective receptor-mediated gene transfer. As far as the molecular design of the galactosylated protein is concerned, we demonstrated that it is recognized by the liver cells according to the estimated surface density of the galactose residues (8). It is likely that the same strategy applies for the liposomes. However, the transfection activity of the liposomes was almost constant when the ratio of Gal-C4-Chol to DC-Chol varied from 2:8 to 8:2 while keeping the amount of DOPE and DNA constant (data not shown). This means that the density of Gal-C4-Chol on the surface of the liposomes for receptor-mediated gene transfer was sufficient at a molar content of 12 % (Gal-C4-Chol/DC-Chol ratio of 2:8).

It has been demonstrated that the spacer length in liposomes bearing specific ligands is critical in binding

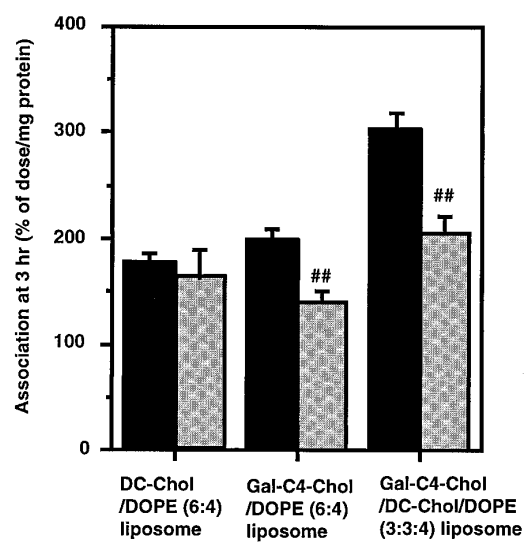

FIG. 6. Inhibitory effect of copresence of 20 mM galactose on cellular association of ^{32}P-labeled DNA complexed with liposomes at 3 h in HepG2 cells at 37°C. Cells were transfected with DNA/liposome complexes in the presence (▨) and absence (■) of 20 mM galactose. DNA concentration was fixed at 0.5 μg/ml in all experiments. DNA/liposome ratios are 1:5 (w/w): DC-Chol/DOPE(6:4), 1:10 (w/w): Gal-C4-Chol/DOPE(6:4), 1:10 (w/w): Gal-C4-Chol/DC-Chol/DOPE(3:3:4), respectively. Each value represents the mean ± S.D. values (n=3). Stastical analysis was performed by analysis of variance ($^{\#\#}P<0.01$).

to the receptors (20-22). In Fig. 4, the inhibition of gene expression by excess galactose, which represents the extent of receptor-mediated gene transfer was greatest with Gal-C6-Chol/DC-Chol/DOPE(3:3:4) liposomes, followed by Gal-C4-Chol/DC-Chol/DC-Chol/DOPE(3:3:4) and Gal-C2-Chol/DC-Chol/DOPE(3:3:4) liposomes. Thus, the longer spacer length resulted in an increased transfection activity of galactosylated liposomes reflecting the higher recognition capacity by asialoglycoprotein receptors.

Remy et al. (23) demonstrated the feasibility of galactose-presenting lipopolyamine vectors towards targeted gene transfer into hepatoma cells. Inclusion of galactose residues in the electrically neutral complex increased transgene expression approaching the value obtained with a large excess of cationic liposomes alone. They stressed that the galactose-presenting DNA particles may avoid interacting serum proteins because of their electric neutrality. However, Yang and Huang (16) have recently reported that DNA/liposome complexes interact with serum even when it is net negative and suggested that excess cationic charge in the complex is required for gene transfer in the presence of serum. In the present study, our galactosylated liposomes had greater transfection activity than cationic liposomes alone, thus suggesting advantage of advantage of our galactosylated cationic liposomes in vivo.

In summary, we synthesized novel galactosylated cholesterol derivatives for developing a hepatocyte-specific gene carrier. In HepG2 cells, the liposomes containing galactosylated cholesterol derivatives showed higher transfection activities than DC-Chol liposomes based on a receptor-mediated mechanism. Since the derivatives themselves have a positive charge, they allow a high density of galactose residues to accumulate on the liposome surface without loosing the binding ability of cationic liposomes to DNA. Moreover, the hydrophilic nature of the galactose residues prevents the DNA/liposome complex from aggregating. These characteristics of liposomes with a galactosylated cholesterol derivative are reflected in their superior in vivo gene transfection potential in the liver in a following paper.

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