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Carbamate-linked cationic lipids with different hydrocarbon chains for gene delivery

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ABSTRACT

A series of carbamate-linked cationic lipids containing saturated or unsaturated hydrocarbon chains and quaternary ammonium head were designed and synthesized. After recrystallization, carbamate-linked cationic lipids with high purity (over 95%) were obtained. The structures of these lipids were proved by IR spectrum, HR-ESI-MS, HPLC, ^{1}H NMR and ^{13}C NMR. The liposomes were prepared by using these cationic lipids and neutral lipid DOPE. Particle size and zeta-potential were studied to show that they were suitable for gene transfection. The DNA-bonding ability of C12:0, C14:0 and C18:1 cationic liposomes was much better than others. The results of transfection showed that hydrophobic chains of these lipids have great effects on their transfection activity. The lipids bearing C12:0, C14:0 saturated chains or C18:1 unsaturated chain showed relatively higher transfection efficiency and lower cytotoxicity. So these cationic lipids could be used as non-viral gene carriers for further studies.

1. Introduction

Gene therapy, mediated by the delivery of genetic materials into target cells to promote or rectify the expression of specific gene, is a promising clinical modality to treat various human diseases, including cancer, infectious diseases, and immunodeficiency [1–6]. However, one of the most difficult hurdles in achieving effective gene therapy is the requirement for the use of efficient vehicles to deliver gene into target cells [7]. Among the non-viral vectors, cationic lipids have been deeply investigated because of their handy synthesis, low immune response, and good safety [8–13]. Cationic lipids interact electrostatically with the negatively charged DNA and condense it into lipid/DNA aggregates, named lipoplexes, which can be taken up by the target cells via an endocytosis mechanism, involving the interaction of residual positive charges on the lipoplexes with negatively-charged cell membrane residues [14]. Spelios et al. [15] synthesized a carbamate-linked cationic lipid (1,3lb2) as siRNA (small interfering RNA) delivery systems to inhibit the production of vascular endothelial growth factor (VEGF) in human prostate carcinoma cell line PC-3. Huang et al. [16] obtained two novel cationic lipids based on protonated cyclen and steroid (cholesterol or diosgenin) moieties with carbamate linkage for gene delivery. Medvedeva et al. [17] synthesized cholesterol-based cationic lipids containing positively charged pyridine and methyl imidazole head groups and ester or carbamate linkers for gene delivery. Meanwhile there are some reports in the literatures focusing on variation of alkyl chain length or unsaturated degree [18–20]. Recently, we have reported the synthesis of carbamate-linked cationic lipids based on a glycerol backbone between the C12 saturated hydrophobic chain and quaternary ammonium headgroup, and investigated the effect of different headgroups on the transfection efficiency and cytotoxicity of cationic liposomes [21,22]. However, we didn't discuss the effect of the chain length and unsaturated degree on gene transfection activities. What's more, there is no clear and unified conclusion about the effect of hydrophobic tails on gene delivery. Therefore, in this article we designed and synthesized five carbamate-linked cationic lipids (Fig. 1), they differed in the length and unsaturated degree of the hydrophobic tail chains, varying from C12:0 to C18:0 or C18:1. We discussed the effect of these tails on gene delivery, and we hope this work will provide insights into the study of relevant non-viral gene delivery vectors.

2. Materials and methods

2.1. Materials

3-Chloro-1,2-propanediol was acquired from Johnson Matthey (Hong Kong, China). Dimethylamine was bought from Sinopharm Holding Co., Ltd. (Shanghai, China). N,N-carbonyldiimidazole (CDI), tetradecylamine, hexadecylamine, octadecylmine and

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oleylamine were purchased from Aladdin Industrial Corporation (Shanghai, China). Fetal bovine sera (FBS) and Cell culture media were purchased from Invitrogen Corporation (Carlsbad, CA, USA). DOPE, Dulbecco's modified Eagle's medium (DMEM), RPMI1640 and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) were obtained from Sigma Co., Ltd. (USA). Lipofectamine 2000 reagent was obtained from Invitrogen Corporation (Shanghai). 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) reagent was from Roche Diagnostics GmbH. pGFP-N2 and pGL3 plasmids vectors were purchased from Sangon Biotech Co., Ltd. (China). Luciferase assay system was purchased from Promega Biotech Co., Ltd. (Beijing, China).

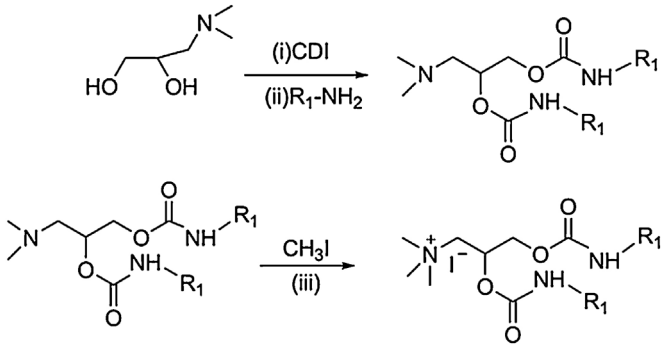
2.2. Synthesis of the intermediates

These lipids were synthesized based on a previously reported method [22]. The synthetic route for intermediates is shown in Scheme 1. A mixture of CDI, 3-(dimethylamino) propane-1,2-diol, and methylbenzene was stirred under nitrogen at 40 °C. After 4 h, amine solution in toluene was added dropwise over 1 h and the reaction mixture was stirred at 40 °C. The solvents were removed in vacuum drying oven to give intermediates as white powder. The yields of intermediates can reach to 72–76%.

2.3. Synthesis of cationic lipids

A mixture of intermediates and iodomethane was stirred in an autoclave reaction vessel at 80 °C for 24 h. After cooling, the product was washed with solvent, and concentrated in vacuum drying oven. Recrystallization was performed to give the final products as white powder. Their yields were from 75% to 84%.

C14:0: IR, (KBr) ν/cm^{-1}: 3459.85 (ν_{NH}), 2919.41 (ν_{CH3}), 2851.39 (ν_{CH2}), 1721.30 ($\nu_{C=O}$), 1518.72 (δ_{NH}), 1136.11 (ν_{COC}); ^{1}H NMR (400 MHz, $CDCl_3$) d: 5.96 (m, 1H, OCH), 5.47–5.20 (d, 2H, 2 × NH), 4.38–4.13 (4H, NH(C=O)OCH_2CH, $CHCH_2$N), 3.48 (s, 9H, $N(CH_3)_3$), 3.21–3.13 (t, 4H, 2 × CH_2NH(C=O)O), 1.58–1.51 (m, 4H, 2 × CH_2CH_2NH), 1.25 (s, 44H, 2 × $(CH_2)_{13}$), 0.89–0.86 (t, 6H, 2 × CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.62–154.54 (C=O), 66.84 (OCH), 66.50 (OCH_2), 63.50 (NCH_2), 55.11 ($N(CH_3)_3$), 41.65–41.44 ($NHCH_2$), 32.15 ($CH_2CH_2CH_3$), 30.06–29.45 (($CH_2)_8$, $NHCH_2CH_2$), 27.16–27.00 ($CH_2CH_2CH_2$NH), 22.91 (CH_2CH_3), 14.34 (CH_3); HR-ESI–MS, *m/z*: Found [M−I] $^+$, 612.5664, $C_{36}H_{74}IN_3O_4$ calcd for [M−I] $^+$, 612.5679.



Scheme 1. Synthetic route of cationic lipids.

Fig. 1. Structures of cationic lipids having different hydrophobic tails.

C16:0: IR, (KBr) ν/cm^{-1}: 3349.74 (ν_{NH}), 2923.30 (ν_{CH3}), 2850.89 (ν_{CH2}), 1708.36 ($\nu_{C=O}$), 1545.25 (δ_{NH}), 1118.81 (ν_{COC}); ^{1}H NMR (400 MHz, $CDCl_3$) d: 6.00(m, 1H, OCH), 5.96–5.45 (d, 2H, 2 × NH), 4.38–4.18 (4H, NH(C=O)OCH_2CH, $CHCH_2$N), 3.50 (s, 9H, $N(CH_3)_3$), 3.15–3.06 (t, 4H, 2 × CH_2NH(C=O)O), 1.64–1.53 (m, 4H, 2 × CH_2CH_2NH), 1.26 (s, 52H, 2 × $(CH_2)_{13}$), 0.98–0.82 (t, 6H,2 × CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.50–155.48 (C=O), 66.97 (OCH), 63.74 (OCH_2), 54.44 (NCH_3), 49.12 ($N(CH_3)_2$), 41.56–41.17 ($NHCH_2$), 34.44 ($CH_2CH_2CH_3$), 32.13–29.90 (($CH_2)_{10}$, $NHCH_2CH_2$), 28.31–27.12 ($CH_2CH_2CH_2$NH), 22.89 (CH_2CH_3), 14.34 (CH_3); HR-ESI–MS, *m/z*: Found [M−I] $^+$, 668.6298, $C_{40}H_{82}IN_3O_4$ calcd for [M−I] $^+$, 668.6305.

C18:0: IR, (KBr) ν/cm^{-1}: 3422.15 (ν_{NH}), 2923.30 (ν_{CH3}), 2850.89 (ν_{CH2}), 1726.65 ($\nu_{C=O}$), 1518.18 (δ_{NH}), 1254.86 (ν_{COC}); ^{1}H NMR (400 MHz, $CDCl_3$) d: 6.02(m, 1H, OCH), 5.47–5.40 (d, 2H, 2 × NH), 4.34–4.11 (4H, NH(C=O)OCH_2CH, $CHCH_2$N), 3.50 (s, 9H, $N(CH_3)_3$), 3.15–3.06 (t, 4H, 2 × CH_2NH(C=O)O), 1.52–1.41 (m, 4H, 2 × CH_2CH_2NH), 1.26 (s, 60H, 2 × $(CH_2)_{15}$), 0.98–0.82 (t, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.81–158.31 (C=O), 66.73 (OCH), 63.63 (OCH_2), 55.45 (NCH_3), 49.12 ($N(CH_3)_2$), 41.70–41.18 ($NHCH_2$), 36.67 ($CH_2CH_2CH_3$), 32.13–29.91 (($CH_2)_{12}$, $NHCH_2CH_2$), 27.81–27.14 ($CH_2CH_2CH_2$NH), 22.89 (CH_2CH_3), 14.34 (CH_3); HR-ESI–MS, *m/z*: Found [M−I] $^+$, 724.6922, $C_{44}H_{90}IN_3O_4$ calcd for [M−I] $^+$, 724.6931.

C18:1: IR, (KBr) ν/cm^{-1}: 3340.23 (ν_{NH}), 3014.00 ($\nu_{C=C}$), 2923.30 (ν_{CH3}), 2850.89 (ν_{CH2}), 1617.66 ($\nu_{C=O}$), 1463.32 (δ_{NH}), 1254.86 (ν_{COC}); ^{1}H NMR (400 MHz, $CDCl_3$) d: 5.98(m, 1H, OCH), 5.67–5.54(m, 4H, CH=CH), 5.48–5.32 (d, 2H, 2 × NH), 4.34–4.12 (4H, NH(C=O)OCH_2CH, $CHCH_2$N), 3.51 (s, 9H, $N(CH_3)_3$), 3.15–3.06 (t, 4H, 2 × CH_2NH(C=O)O), 2.11–1.85 (m, 8H, 2 × CH_2CH=$CHCH_2$), 1.64–1.53 (m, 4H, 2 × CH_2CH_2NH), 1.27 (s, 44H, 2 × $(CH_2)_{11}$), 1.00–0.88 (t, 6H, 2 × CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.85–154.78 (C=O), 130.17–129.93 (HC=CH), 66.99 (OCH), 66.34 (OCH2), 55.09 $N(CH_3)_3$, 41.62–41.42 ($NHCH_2$), 32.81 (CH_2CH=$CHCH_2$), 32.12 ($CH_2CH_2CH_3$), 29.91 $(CH_2)_4CH_2$CH=$CHCH_2(CH_2)_4$, 27.81–27.14 ($CH_2CH_2CH_2$NH), 22.88 (CH_2CH_3), 14.30 (CH_3); HR-ESI–MS, *m/z*: Found [M−I] $^+$, 720.6639, $C_{44}H_{86}IN_3O_4$ calcd for [M−I] $^+$, 720.6619.

2.4. Preparation of liposomes

A solution of cationic lipid (1 mg) in chloroform (1 mL) and DOPE (n_{lipid}/n_{DOPE} = 1/1) was mixed and evaporated under a stream of nitrogen, and the residual solvent was removed under vacuum for 5 h. Liposomes were prepared by resuspending the lipids in distilled water (1 mL) at 55 °C and sonicating at the temperature for 2 h in a vial.

2.5. Gel electrophoresis assay

To characterize the electrostatic binding interactions between the plasmid DNA and the cationic liposomes as a function of N/P weight ratios, the electrophoretic gel retardation assays was performed. Cationic liposomes were mixed with pDNA in DMEM at N/P ratios from 0.5/1 to 8/1 and incubated for 30 min at room temperature. Each mixture was loaded onto a 1.2% agarose gel containing 0.5 mg/mL ethidium bromide. The samples were subjected to electrophoresis at 90 V for 30 min, and DNA bands were visualized in a gel documentation unit.

2.6. Particle size and zeta potential

The liposome solutions were subjected to several cycles of sonication in a bath sonicator and vigorous vortex mixing to form small vesicles. For the measurement of the particle size and zeta potential, 20 μL of the liposomes were diluted with distilled water (1 mL).