In this work two novel cationic lipids using natural tartaric acid as linking backbone were synthesized. These cationic lipids were simply constructed by tartaric acid backbone using head group 6-aminocaproic acid and saturated hydrocarbon chains dodecanol (T-C12-AH) or hexadecanol (T-C16-AH). The physicochemical properties, gel electrophoresis, transfection activities, and cytotoxicity of cationic liposomes were tested. The optimum formulation for T-C12-AH and T-C16-AH was at cationic lipid/dioleoylphosphatidylethanolamine (DOPE) molar ratio of 1:0.5 and 1:2, respectively, and N/P charge molar ratio of 1:1 and 1:1, respectively. Under optimized conditions, T-C12-AH and T-C16-AH showed effective gene transfection capabilities, superior or comparable to that of commercially transfecting reagent 3β-[N-(N',N'-dimethylaminoethyl)carbamoyl]cholesterol (DC-Chol) and N-[2,3-dioleoyloxypropyl]-N,N,N-trimethylammonium chloride (DOTAP). The results demonstrated that the two novel tartaric acid-based cationic lipids exhibited low toxicity and efficient transfection performance, offering an excellent prospect as nonviral vectors for gene delivery.

**Key words** nonviral vector; cationic lipid; tartaric acid; gene transfection

Nowadays, gene therapy is considered to be a powerful approach in curing a multitude of diseases, especially in developing strategies for the prevention and treatment of many diseases, such as cancer, AIDS and so on.1,2) The vectors used for gene transfection play the key role in successful gene therapy, which are roughly divided into viral and nonviral ones.3–5) Viral vectors showed high transfection efficiency for gene delivery,6,7) but the huge disadvantages of viral vectors limited their clinical applications, such as immunogenic responses, high cost of production and limitation of the exogenous DNA size.8–13)

Since the application of viral vectors are limited, nonviral vectors including cationic lipids, cationic polymers have been drawing more and more attention nowadays.14–24) Among the existing nonviral vectors, the cationic liposome, representing an attractive, alternative approach for gene delivery has a broad variety of advantages, such as biodegradability, easy preparation, good repeatability and potential clinical applications.25–29)

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Cationic lipids generally consist of polar head group and hydrophobic tails connected through the backbone (Fig. 1), while most of the backbone in earlier cationic lipids was classified into glycerol-type just as N-[2-[(1,5,10,14-tetraazatetradecane-1-yl)carboxylaminoethyl]-N,N,N-dimethyl-2,3-bis(oxyoloyloxy)-1-propanaminium (DOSPA) and N-[2,3-dioleoyloxypropyl]-N,N,N-trimethylammonium chloride (DOTAP).30,31) and cholesterol-type, such as 3β-[N-(N',N'-dimethylaminoethyl)carbamoyl]cholesterol (DC-Chol).32) Recently, phosphonate,33) bile acid,34) amino acids,35,36) peptides,37) pentaerythritol38) and carbohydrate39) were also successfully used as backbones in the design of cationic lipids, which can give a favorable gene delivery efficiency and low cytotoxicity.

Natural tartaric acid is inexpensive and readily available and widely used in drinks and other foods.40) Tartaric acid, a multi-functional molecule, has two hydroxyl and two carboxyl groups as reactive sites, which is suitable as the backbone in the design of cationic lipids through easily modified with different head group and alkyl hydrophobic tail.

In this paper, natural tartaric acid was used as a backbone to design and synthesize the cationic lipids for gene delivery. The cationic lipids were simply constructed by tartaric acid backbone using polar head group 6-aminocaproic acid and saturated hydrocarbon chains dodecanol (T-C12-AH) or hexadecanol (T-C16-AH) (Fig. 1). The liposome formulations for gene delivery were prepared and optimized by introducing helper lipid dioleoylphosphatidylethanolamine (DOPE) at various cationic lipid/DOPE ratios and cationic lipid/DNA ratios. The cytotoxicity of the cationic liposomes was also evaluated.

**MATERIALS AND METHODS**

**Materials** Natural tartaric acid, dodecanol, hexadecanol, 6-aminocaproic acid (AH), di-tert-butyl dicarbonate ((Boc),O), dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA) and 4-dimethylaminopryidine (DMAP) were purchased from Sun Chemical Technology (Shanghai) Co., Ltd. All starting materials and reagents were used without further purification. Silica gel (300–400 mesh) and potassium bromide (KBr, spectroscopic grade) were purchased from Xi’an Ke Hao Biological Engineering Co., Ltd. (China). DOPE was bought from Fluka (Buchs, Switzerland). DC-Chol was bought from Sigma (St. Louis, MO, U.S.A.). pEGFP-N1 encoding the enhanced green fluorescence protein (GFP) was purchased from Shang-
hai GenePharma Co., Ltd. (Shanghai, China). Fetal bovine serum (FBS), opti-minimal essential media (MEM) and Dulbecco’s modified Eagle’s medium (DMEM) were bought from Gibco (Carlsbad, CA, U.S.A.).

Cells HEK 293T cells and HeLa cells were bought from the Culture Collection of the Chinese Academy of Science (Shanghai, China). The cells were grown in DMEM with 10% FBS at 37°C in 5% CO₂, penicillin at 100 U mL⁻¹ and streptomycin at 100 µg mL⁻¹.

Characterization of the Synthesized Compounds TLC was performed to test the reactions. Purification was carried out by silica gel column chromatography. IR spectra were recorded using a Fourier transform (FT)-IR spectrometer. All samples to be tested were ground and compressed with KBr into a thin disk under hydraulic press. ¹H-NMR spectra were recorded at 400 MHz. Mass spectra were detected by Quattro Premier Micromass.

Synthesis of the Cationic Lipids T-C₁₂-AH and T-C₁₆-AH

Synthesis of Boc-AH 6-Aminocaproic acid (2.00 g, 15.25 mmol) was dissolved in 40 mL NaOH solution (0.62 mol/L). Then (Boc)₂O (3.66 g, 16.77 mmol) in 22 mL tetrahydrofuran (THF) was added dropwise into the flask at 0°C. The mixture was stirred for 30 min at 0°C and then reacted at room temperature for 24 h. A rotary evaporator was used to remove the THF. Then 100 mL Et₂O was applied to extract the unreacted (Boc)₂O. One mol/liter HCl was added dropwise into the aqueous phase until the pH was approximately 3. The aqueous phase was then extracted with 150 mL CH₂Cl₂ and washed with deionized water for 3 times. Organic solvent was evaporated under reduced pressure and the product was dried in a vacuum oven (45°C) to afford yellowish thick liquid, 2.13 g, yield: 61.6%. MS m/z electronspray ionization (ESI)⁺: 232 (M)⁺. IR (KBr) cm⁻¹: 3348, 2978, 2939, 2719, 2646, 1670, 1651, 1504, 1273, 1173, 864, 779. ¹H-NMR (CDCl₃) δ: 1.38–1.34 (2H, t, J= 7.8 Hz), 1.43 (9H, s), 1.53–1.47 (2H, m), 1.68–1.61 (2H, m), 2.37–2.33 (2H, t, J= 7.4 Hz), 3.12–3.09 (2H, t, J= 6.1 Hz), 4.56–4.55 (1H, d, J=0.6Hz).

Synthesis of Compound 2 Tartaric acid (1, 4.90 g, 32.65 mmol), dodecanol (13.38 g, 71.83 mmol) and concentrated hydrochloric acid (0.7 mL) were added into a round-bottom flask. The reaction solution was heated under stirring for 36 h at 120°C. The mixture was then cooled to room temperature. The obtained white solid was rinsed by 1 mol/L sodium hydrate solution (3 × 6 mL) and a small amount of water. Then the resulting solid was recrystallized for twice times from ethanol to give compound 2, 11.40 g, white solid, yield: 71.7%, mp: 65–66°C [62–64°C (41)]. MS m/z ESI⁺: 488 (M)⁺. IR (KBr) cm⁻¹: 3445, 2916, 2847, 1720, 1635, 1277, 1103, 1068, 876, 748. ¹H-NMR (CDCl₃) δ: 0.89–0.86 (6H, t, J=5.1 Hz), 1.26 (36H, m), 1.70–1.65 (4H, m), 4.27–4.23 (4H, m), 4.52 (1H, s).

Synthesis of Compound 3 Tartaric acid (1, 4.90 g, 32.65 mmol), hexadecanol (17.41 g, 71.83 mmol) and concentrated hydrochloric acid (0.7 mL) were added into the 100 mL round-bottom flask. The reaction solu-
tion was heated under stirring for 24 h at 120 °C. Then the mixture was cooled to room temperature, and the obtained white solid was rinsed by 1 mol/L sodium hydrate solution (3×6 mL) and a small amount of water, recrystallized from ethanol two times to give compound 3, 14.31 g, white solid, yield: 73.2%, mp: 78–79°C. IR (KBr) cm⁻¹: 3418, 2916, 1850, 1751, 1682, 1273, 1203, 1134, 1076, 841, 741. ¹H-NMR (CDCl₃) δ: 0.89–0.86 (6H, t, J = 6.8 Hz), 1.25 (52H, s), 1.70–1.67 (4H, t, J = 7.2 Hz), 4.28–4.24 (4H, m), 4.52 (2H, s).

Synthesis of Compound 4

DCC (0.76 g, 3.70 mmol), DMAP (4 mg, 34.4 µmol) and compound 2 (0.30 g, 0.62 mmol) were added to a solution of Boc-ACH (0.57 g, 2.47 mmol) in dry CH₂Cl₂ (10 mL) at 0°C stirring for 1 h. Then the mixture was stirred for another 5 h at room temperature. After filtering, the filtration was evaporated under reduced pressure, then the resulting residue was further purified by column chromatography (petroleum ether (PE): EtOAc, 5:1) to give compound 4, 0.32 g, white solid, yield: 85.3%, mp: 62–63°C. MS m/z ESI⁺: 914 (M⁺). IR (KBr) cm⁻¹: 3375, 2924, 2850, 1770, 1686, 1516, 1250, 1157, 1041, 999, 868, 729. ¹H-NMR (CDCl₃) δ: 0.89–0.86 (6H, t, J = 6.4 Hz), 1.25–1.22 (36H, m), 1.35–1.33 (4H, m), 1.43 (18H, m), 1.67–1.60 (12H, m), 2.45–2.31 (4H, m), 3.11–3.10 (4H, d, J = 2.4 Hz), 4.16–4.13 (4H, t, J = 7.4 Hz), 4.62 (2H, s), 5.70 (2H, s).

Synthesis of Compound 5

DCC (0.84 g, 4.13 mmol), DMAP (4 mg, 34.4 µmol) and compound 3 (0.41 g, 0.69 mmol) were added to a solution of Boc-ACH (0.64 g, 2.75 mmol) in dry CHCl₃ (10 mL) at 0°C and stirred for 1 h. Then the mixture was stirred at room temperature for another 5 h. After filtering, the filtration was evaporated under reduced pressure, then the resulting residue was further purified by column chromatography (PE: EtOAc, 6:1) to give compound 5, 0.64 g, White solid, yield: 91.2%, mp: 45–46°C. MS m/z ESI⁺: 1026 (M⁺). IR (KBr) cm⁻¹: 3402, 2924, 2854, 1751, 1716, 1701, 1273, 1250, 1173, 1150, 868, 721. ¹H-NMR (CDCl₃) δ: 0.89–0.86 (6H, t, J = 6.4 Hz), 1.25 (52H, m), 1.40–1.34 (4H, m), 1.51–1.43 (18H, m), 1.68–1.60 (12H, m), 2.45–2.38 (4H, m), 3.13–3.09 (4H, m), 4.16–4.11 (4H, m), 4.61 (2H, s), 5.70 (2H, s).

Synthesis of T-C12-AH

Trifluoroacetic acid (1 mL) was added to the solution of compound 4 (0.38 g, 0.42 mmol) in CH₂Cl₂ (10 mL) at 0°C for 30 min, and reacted for another 4 h at room temperature. Then CH₂Cl₂ was evaporated under reduced pressure, and the residue was purified by column chromatography (CH₂Cl₂: MeOH:H₂O, 80:10:1) to obtain T-C12-AH 0.43 g, white solid, yield: 77.3%, mp: 51–53°C. MS m/z ESI⁺: 826 (M–CF₃COOH–CF₂COO)⁻, 413 (M–2CF₂COO)⁻². IR (KBr) cm⁻¹: 3418, 2916, 1850, 1751, 1682, 1273, 1203, 1134, 1076, 841, 741. ¹H-NMR (CDCl₃) δ: 0.89–0.86 (6H, t, J = 6.8 Hz), 1.25 (s, 52H), 1.48–1.45 (4H, m), 1.78–1.61 (12H, m), 2.96–2.94 (4H, t, J = 7.2 Hz), 2.43–2.39 (4H, m), 4.16–4.13 (4H, t, J = 6.6 Hz), 5.67 (2H, s), 7.87 (6H, s).

Preparation of Liposomes and Lipoplexes

Liposomes were prepared through thin-film hydration method. DOPE and lipids were taken in desired molar ratios and dissolved in appropriate amount of chloroform, solvent was slowly removed under vacuum. The resulting film was placed in vacuum oven (45°C) overnight and then hydrated in deionized water to the final cationic lipid concentration of 1 mm. Hydration process continued at 4°C for 12 h. The hydration solution was vortex-mixed for about 5 min and sonicated for another 15 min. Liposomes were then extruded through filter with porosity of 0.45 µm and 0.2 µm for six times respectively and stored at 4°C. Lipoplexes were prepared as followed. The cationic liposomes diluted with an appropriate amount of opti-MEM were mixed with pEGFP-N1 diluted with dd H₂O. The mixture was then gently vortexed and incubated for 30 min at room temperature to form lipoplexes (cationic liposome/DNA complexes). The lipoplexes were then diluted with an appropriate amount of opti-MEM for analyzing the gene transferring efficiency. Cationic liposome/DNA lipoplexes were prepared at a DNA concentration of 25 µg/mL for the measurement of particle size and zeta potential. The lipoplexes formed by the liposomes of DOTAP or DC-Chol/DOPE (1:1, molar ratio) with plasmid DNA (pDNA) at the optimal N/P ratio of 1:1 were used as the positive control groups.

Measurement of Size Distribution and Zeta-Potential of Cationic Liposomes and Lipoplexes

The particle size and zeta potential of cationic liposomes and cationic liposome/DNA lipoplexes were determined with the Delsa™ Nano C Particle Analyzer (Beckman Coulter) by the dynamic light scanning method, and were determined for 3 times. Data were analyzed using the ELS-Z software package supplied by the manufacturer.

Gel Retardation Assay

The gel electrophoresis assay was carried out to evaluate the electrostatic interactions and optimize the lipid/DNA ratios between cationic lipids and DNA. In brief, 0.8 µg pDNA was mixed with liposomes at different N/P ratios. The ethidium bromide intercalating agent was used as staining reagent. Electrophoresis was performed in 0.5× Tris borate ethylenediaminetetraacetic acid (TBE) running buffer at 100 V for 50 min. The gel images were taken by a UV light illuminator.

Transfection Activity

The DNA delivery efficiency of synthesized cationic lipids was determined in 293T cells using GFP as a reporter gene. The transfection activities of the cationic liposomes were tested by flow cytometry and fluorescence microscopy.

As for flow cytometry assay, 293T cells or HeLa cells were seeded on 6-well plates at 20000 cells/well in DMEM containing 10% FBS for 24 h before transfection. The cells were rinsed with DMEM (1 mL) and another 800 µL serum free DMEM was added. Two hundred microliters of the lipoplex formulations (containing 2.5 µg DNA) in opti-MEM were added to cells, and incubated for 8 h at 37°C. Transfection media was then replaced by 2 mL of DMEM containing 10%
FBS, and the cells were incubated for another 40 h. The cells were collected for flow cytometry using a Becton and Dickinson flow cytometer. Both the mean fluorescence intensity (MFI) and the percent of positive transfected cells had been recorded for transfection activity evaluation.

When tested by fluorescence microscopy, 293T cells were plated at 24-well plates at 50000 cells/well, and incubated for 24 h. The desired lipid formulation of different N/P ratios and DNA (0.8 µg per well) were complexed in opti-MEM and incubated for 30 min at room temperature. Next, original cell cultures were discarded, and cells were rinsed once with DMEM. Then, the lipoplexes mentioned above were added to the cells. After incubation at 37°C for 8 h, transfaction media were removed, and replaced by 500 µL DMEM containing 10% FBS. The transfection was stopped after incubation for another 40 h. Fluorescence microscopy was applied to examine GFP expression.

Cytotoxicity Assay The cytotoxicity of optimized formulation of cationic liposomes was also investigated with 293T cells. Briefly, cells were seeded on 96-well plates at 20000 cells/well, and incubated for 24 h under 5% CO2 at 37°C and cultured with 200 µL of DMEM containing 10% FBS. The cytotoxicity assay was carried out to evaluate the binding interactions between cationic liposomes and pDNA at different N/P ratios. The yield of compounds 2 and 3 after recrystallization was 71.7 and 73.2%, respectively. The protected 6-aminocaproic acid derivative Boc-AH21 was reacted with the compounds T-C12-AH and T-C16-AH via an amide linkage to synthesize compounds 4 or 5. It was worth noting that compound 2 had better solubility in CHCl3 than CH2Cl2, so we chose CHCl3 as the solvent for compound 2. After deprotection with trifluoroacetic acid, two novel tartaric acid based cationic lipids were obtained.

RESULTS AND DISCUSSION

Synthesis of the Cationic Lipids T-C12-AH and T-C16-AH The cationic lipid materials T-C12-AH and T-C16-AH were constructed as shown in Fig. 2. The double chain hydrocarbons are normally ranging from 12 to 18 carbon units in length,20 so dodecanol and hexadecanol were used and esterified to the carboxyl groups of tartaric acid to prepare cationic lipid in three steps with high yield and the effect of hydrophobic chain length on the transfection efficiency was examined. The yield of compounds 2 and 3 after recrystallization was 71.7 and 73.2%, respectively. The protected 6-aminocaproic acid derivative Boc-AH21 was reacted with the compounds T-C12-AH and T-C16-AH via an amide linkage to synthesize compounds 4 or 5. It was worth noting that compound 2 had better solubility in CHCl3 than CH2Cl2, so we chose CHCl3 as the solvent for compound 2. After deprotection with trifluoroacetic acid, two novel tartaric acid based cationic lipids were obtained.

Preparation and Characterization of Cationic Liposomes Cationic lipids were often mixed with a neutral co-lipid dioleoylphosphatidylethanolamine (DOPE) to formulate into cationic liposomes.22–27 The results of the particle size, size distribution and zeta potential of the cationic liposomes were shown in Table 1. The cationic formulation of two cationic lipids at lipid/DOPE ratio of 1:1 exhibited rather small hydrodynamic diameters 78.2 nm for T-C12-AH and 83.7 nm for T-C16-AH. The positive potential was 58.6 and 58.4 mV, respectively. These results indicated that the hydrophobic domain of cationic lipids exerted little influence on the physical properties of liposomes.

Agarose Gel Retardation Assay Agarose gel retardation assay was carried out to evaluate the binding interactions between cationic liposomes and pDNA at different N/P ratios.
Stable cationic liposome/DNA complexes were not able to penetrate into the agarose gel, in contrast, free DNA or DNA not fully combined to cationic liposomes could penetrate into the agarose gel. As shown in Fig. 3, between N/P ratio of 1 : 4 and 5 : 1, compared with free DNA, the free mobile DNA disappeared at N/P ratio of 1 : 1 (cationic lipid/DNA molar ratio of 0.5 : 1) for both cationic lipids T-C12-AH and T-C16-AH. In addition, when N/P ratio was over 1 : 1, DNA was entirely compacted and protected in the cationic liposomes, the fluorescent in the corresponding lanes was absence because the staining reagent was inaccessible to stain DNA.35,48)

Optimization of Cationic Lipid/DOPE Ratios Neutral helper lipid DOPE plays an important role to form cationic liposomes. The appropriate addition of DOPE has been reported to increase the gene delivery efficiency of cationic liposomes.49–51) In an effort to research the influence of cationic lipid/DOPE ratios on the capacity of gene delivery efficiency, we prepared a series of cationic liposomes with different cationic lipid/DOPE molar ratios of 1 : 0.5, 1 : 1, 1 : 2, and 1 : 3 at equal lipid/DNA molar ratio of 3 : 1. The transfection activity was evaluated through the percent of positive transfected cells and MFI by flow cytometry in 293T cells as shown in Fig. 4. The optimized lipid/DOPE ratio was found to be different for the two cationic lipids. When the ratio of DOPE increased from 0.5 to 2, the gene delivery efficiency of lipid T-C16-AH increased. However, the gene delivery efficiency reduced with the increase of the ratio of DOPE to 3. At lipid/DOPE ratio of 1 : 2, the MFI of T-C16-AH liposome was highest among others. While for lipid T-C12-AH, the transfection activities reduced with the increase of DOPE ratio and the optimal lipid/DOPE ratio was 1 : 0.5. Although the MFI of lipid T-C12-AH at lipid/DOPE ratio of 1 : 1 was close to that of at lipid/DOPE ratio of 1 : 0.5, the percent of positive transfected cells was lower. The images of GFP expression observed by a fluorescence microscope were in line with the aboved results (Fig. 5). Taking the percent of positive transfected cells and MFI into consideration, we finally chose lipid/DOPE ratio of 1 : 0.5 for T-C12-AH and lipid/DOPE ratio of 1 : 2 for T-C16-AH for further study.

Optimization of Cationic Lipids/DNA Ratios After the optimization of cationic lipid/DOPE ratio, we then determined the particle size and zeta potential of cationic liposomes/DNA lipoplexes and the transfection performance at different N/P ratios to investigate the effect of N/P ratio on transfection. As shown in Table 2, after binding with DNA at N/P ratio of 1 : 1, the particle size of cationic liposomes/DNA lipoplexes increased when compared with free cationic liposomes, from 95.4±1.0 nm to 132.9±1.6 nm (T-C12-AH), and 79.6±1.1 nm to 195.4±0.9 nm (T-C16-AH), respectively. With the increase of cationic lipids (N/P ratios from 1 : 1 to 4 : 1), the particle size of lipoplexes became smaller and more compact because highly tight lipoplexes were formed with the increasing of N/P.

Fig. 3. Agarose Gel Retardation of DNA Lipoplexes of T-C12-AH (A) and T-C16-AH (B) at Various N/P Ratios

Fig. 4. Effects of DOPE on the Transfection Activity of T-C12-AH (A) and T-C16-AH (B) in 293T Cells

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We also measured the zeta potential of these lipoplexes and the control formulations. The lipoplexes showed negative zeta potentials for cationic lipids T-C12-AH or T-C16-AH at N/P ratio of 1, while zeta potentials turned to positive value at N/P ratio over 2. With the increase of cationic lipids, there were excess cationic lipids in the lipoplexes, thus resulting in the increasing of zeta potential. Under the optimal condition of DC-Chol and DOTAP, the formulations also showed the negative zeta potentials.

Lipoplexes at different N/P charge ratios were prepared and examined for transfection activity in 293T cells. The results showed that both T-C12-AH and T-C16-AH at the N/P ratio of 1:1 showed highest gene transfection performance. Both cellular internalization and intracellular DNA release are common barriers for nonviral gene delivery system. Although positively charged lipoplexes are useful in the initial stages of endocytosis, complexes unpacking is generally assumed to be necessary for DNA release and gene expression. The lipoplexes with negative charge at the N/P ratio of 1:1 showed high transfection efficiency because the lipoplexes at the higher N/P ratio compacted DNA too tightly to release DNA into cytoplasm for further gene expression. The lipoplexes formed at high N/P ratio exhibited lower gene transfection performance than that at N/P ratio of 1:1. This can also be confirmed by the results of agarose gel retardation assay, as shown in Fig. 3 and mentioned above, when N/P ratio was over 1:1, DNA was tightly compacted in the cationic liposomes, and the staining reagent was inaccessible to stain DNA thus leading to the absence of the fluorescence in the corresponding lanes. The percent of positive transfected cells of T-C12-AH (47.7%) was better than that of T-C16-AH (32.9%) at N/P ratio of 1:1, while the MFI showed no significant difference between these two lipids at N/P ratio of 1:1 (Fig. 6). It was worth noting that T-C12-AH exhibited higher gene transfection performance in the percent of positive transfected cells than DC-Chol (15.8%) at N/P ratio of 1:1 and 2:1, while showed comparable MFI with DOTAP at N/P charge ratios. T-C12-AH also exhibited higher gene transfection performance in the percent of positive transfected cells than DOTAP (24.7%) at N/P ratio of 1:1 and 2:1, while showed comparable MFI with DOTAP at N/P ratio of 1:1, 2:1 and 3:1. For lipid T-C16-AH, it showed higher gene transfection activity in the percent of positive transfected cells than DC-Chol only at N/P ratio of 1:1, while it exhibited higher MFI than DC-Chol at any N/P charge ratios. T-C16-AH also exhibited higher gene transfection performance in the percent of positive transfected cells than DOTAP at N/P ratio of 1:1, and showed comparable MFI with DOTAP at N/P charge ratios of 1:1 to 4:1. The images of GFP expression observed by a fluorescence microscope were in line with the above results.

| Formulation | Liposome/DNA (molar ratio) | Size (nm) | Polydispersity (PDI) | Zeta potential (mV) |
|-------------|---------------------------|----------|---------------------|---------------------|
| T-C12-AH    | 1:0                       | 95.4±1.0 | 0.258±0.010         | 68.1±0.5            |
|             | 1:1                       | 132.9±1.6| 0.235±0.008         | −17.8±0.2           |
|             | 2:1                       | 123.7±0.3| 0.181±0.010         | 25.4±0.9            |
|             | 3:1                       | 110.9±0.7| 0.196±0.005         | 28.0±1.6            |
|             | 4:1                       | 106.8±1.1| 0.192±0.013         | 34.6±0.5            |
| T-C16-AH    | 1:0                       | 79.6±1.1 | 0.218±0.003         | 61.9±4.4            |
|             | 1:1                       | 195.4±0.9| 0.231±0.014         | −13.3±0.5           |
|             | 2:1                       | 124.0±0.4| 0.134±0.006         | 39.9±2.2            |
|             | 3:1                       | 111.9±1.1| 0.154±0.011         | 41.6±0.9            |
|             | 4:1                       | 103.6±0.2| 0.163±0.018         | 41.7±2              |
| DC-Chol     | 1:0                       | 146.9±1.2| 0.306±0.009         | 58.4±0.4            |
|             | 1:1                       | 173.3±1.2| 0.190±0.012         | −38.5±0.6           |
| DOTAP       | 1:0                       | 100.2±1.2| 0.252±0.002         | 60.4±0.7            |
|             | 1:1                       | 146.8±3.7| 0.236±0.023         | −19.1±3.9           |

Fig. 5. GFP Expression of T-C12-AH (A) and T-C16-AH (B) with Different Lipid/DOPE Molar Ratios in 293T Cells Observed by Fluorescence Microscope
These results indicated that appropriate N/P ratio was important for gene delivery efficiency.

The transfection activity of T-C12-AH and T-C16-AH was also evaluated in HeLa cells at the optimized condition. As shown in Fig. 8, although the MFI of T-C12-AH was lower than those of DC-Chol and DOTAP, T-C12-AH showed higher gene transfection performance in the percent of positive transfected cells than DOTAP and comparable gene transfection performance with DC-Chol. T-C16-AH showed lower gene transfection performance both in the percent of positive transfected cells and MFI than that of DC-Chol, and exhibited comparable gene transfection performance in the percent of positive transfected cells with DOTAP. T-C12-AH still showed higher gene transfection performance in the percent of positive transfected cells than that of T-C16-AH in HeLa cells. Considering the gene delivery efficiency both in 293T cells and HeLa cells, cationic lipid T-C12-AH showed superior transfection activity.

Cytotoxicity Since good biocompatibility is of great importance for gene carriers, the toxicity of cationic lipids T-C12-AH and T-C16-AH was determined. The results were shown as the percent of cell viability compared to the control group. As shown in Fig. 9, both of these two lipids showed lower toxicity than that of DC-Chol (IC$_{50}$=49.9 µM)
and DOTAP (IC50=58.6 µM). The cationic lipids T-C12-AH and T-C16-AH showed low cytotoxicity with IC50=94.5 µM and IC50>100 µM, respectively. The possible mechanism of the difference in delivery efficiency and cytotoxicity between T-C12-AH and T-C16-AH maybe that T-C12-AH with shorter hydrocarbon chains increases the fluidity of the lipid bilayer and promotes the intermembrane transfer of lipid monomers and lipid membrane mixing, thus resulting in more potential disruption of the cell membrane than that of T-C16-AH.28 The cytotoxicity of cationic lipid/DNA complexes was also evaluated and the results indicated that there is no cytotoxicity under the highest gene expression condition for T-C12-AH (1:0.5) and T-C16-AH (1:2) at N/P ratio of 1. These results proved that the synthesized lipids were both safe to use and showed lower toxicity than DC-Chol and DOTAP.

In conclusion, two novel DNA carriers based on natural tartaric acid backbone were synthesized. The results of gel electrophoresis, transfection activities, and cytotoxicity of cationic liposomes prepared with the cationic lipids showed that the tartaric acid based cationic lipids are promising candidates for gene delivery. The newly synthesized cationic lipids T-C12-AH and T-C16-AH displayed efficient transfection performance and less toxicity than commercially available transfecting reagent DC-Chol and DOTAP. Among them, cationic lipid T-C12-AH displayed more sufficient transfection efficiency, which makes it as a potential nonviral gene delivery vector.

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Conflict of Interest The authors declare no conflict of interest.

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