Colloids and Surfaces B: Biointerfaces 112 (2013) 537–541

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Short communication

Structure–activity relationship of carbamate-linked cationic lipids bearing hydroxyethyl headgroup for gene delivery

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ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form 3 August 2013
Accepted 26 August 2013
Available online 1 September 2013

Keywords:
Carbamate-linked cationic lipids
Hydroxyl group
Gene delivery
Transfection efficiency
Cytotoxicity

ABSTRACT

A novel series of carbamate-linked cationic lipids containing hydroxyl headgroup were synthesized and included in formulations for transfection assays. The DNA–lipid complexes were characterized for their ability to bind DNA, their size, ζ-potential and cytotoxicity. Compared with our previously reported cationic transfection lipid DDCDMA lacking the hydroxyl group and the commercially available, these cationic liposomes exhibited relatively higher transfection efficiency.

1. Introduction

Since the initial pioneering work of Felgner et al. [1] in 1987, many new cationic lipids of different structures have been synthesized and their respective application for gene delivery have been published by many reviews [2,3]. The chemical structure of cationic lipids is an important property rendering its application in many areas of research. Publications based on cationic lipids have grown tremendously in the last decade with reports on establishment of a structure–activity relationship to define the requirements for safe and efficient gene transfer with cationic lipids [4–7]. Recently, we described the gene transfection properties of carbamate-linked cationic lipids with C_{12} aliphatic chains (DDCTMA and DDCEDMA), which displayed relatively high transfection efficiency and low toxicity [8]. Herein, the aim of this study was to extend the previous work on cationic lipids as non-viral vectors by investigating a series of new carbamate-linked cationic lipids containing hydroxyethyl group. The idea for the preparation of these new cationic lipids is mainly based on the following characteristics: First, the new compounds are easy to produce starting from environmentally friendly materials. Second, it is preferred that the compounds demonstrate favorable biocompatibility due to the carbamate bond as a connection between quaternary ammonium headgroups and hydrocarbon chains. Third, the incorporation of hydroxyl group may result in the favorable hydrogen-bonding interactions between the lipid headgroups and the cell surface of biological membranes, thereby improving the transfection efficiency in cationic lipid-mediated gene delivery.

2. Materials and methods

2.1. General procedures and materials

Most chemicals were obtained from Sinopharm Holding Co. Ltd. (Shanghai, China). 3-Chloro-1,2-propanediol was purchased from Johnson Matthey (Hong Kong, China). N,N-carbonyldiimidazole (CDI) was purchased from Medpep Co. Ltd. (Shanghai, China). Cell culture media and fetal bovine sera (FBS) were purchased from Invitrogen Corporation (Carlsbad, CA, USA). DOPE, Dulbecco's modified Eagle's medium (DMEM) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Co. Ltd. (USA). Lipofectamine 2000 reagent was purchased from Invitrogen Corporation (Shanghai). DOTAP reagent was purchased from Roche Diagnostics GmbH.

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http://dx.doi.org/10.1016/j.colsurfb.2013.08.040

2.2. Synthesis of 1-dimethylamino-2,3-propanediol (DAP)

33% dimethylamine aqueous solutions (0.5 mol) and 40% NaOH (0.2 mol) aqueous solutions were added to a 250 mL round-bottom flask fitted with magnetic stirrer. 3-chloro-1,2-propylene glycol liquid (0.2 mol) was added drop-wise to the mixture about 1 h. The reaction mixture was heated to 50 °C for a further 4 h. The remaining water and dimethylamine were removed by atmospheric distillation. After filtration, the 117–118 °C/15 mmHg distillates were collected by reduced pressure distillation.

2.3. Synthesis of cationic lipids

N,N-dimethyl-(2,3-didodecyl carbamoyloxy)propylamine (DDCDMA) was prepared according to a previously reported method [8]. DDCDMA (3 mmol) and halogenated hydroxyl hydrocarbons (10 mL) were added into a 40 mL pressure apparatus. The autoclave was sealed tightly, and heated at 80–90 °C for 24 h. After cooling to 0 °C, the reaction vessel was opened, and the excess halogenated hydrocarbons were allowed to evaporate. The crude residue was recrystallized from acetonitrile/alcohol to the final sample.

2.4. Preparation of liposomes

A solution of cationic lipid (1 mg) in chloroform (1 mL) was evaporated under a stream of nitrogen, and the residual solvent was removed under vacuum overnight. Liposomes were prepared by resuspending the lipids in distilled water (1 mL) at 55 °C and sonicating them to clarity at this temperature for 2 h in a closed vial.

2.5. DNA binding assay

DNA–lipid complexes were formed by mixing 1.0 μg of plasmid DNA with varying amounts of cationic lipids so that the final lipid/DNA charge ratios were maintained at 0.5/1–8/1 in a total volume of 30 μL. Complexes were incubated for 30 min at room temperature after which 20 μL of each lipoplex was loaded on a 1.2% agarose gel and subjected to electrophoresis. The samples were electrophoresed at 90 V for 1 h, and the bands were visualized with ethidium bromide staining.

2.6. Transfection assay

Human cervical cancer (HeLa) cells and Human epithelial type 2 (HEp-2) cells were obtained from ATCC (American Type Culture Collection Shanghai Representative Office) and seeded in 100 μL of growth medium (DMEM) without antibiotics. The cells were plated at a density of 1.5×10^4 cells/well in 96-well plate at 37 °C with 5% CO_2 atmosphere and incubated for 24 h. Liposomes and 0.5 μg plasmid DNA were diluted in 25 μL DMEM without serum, respectively, and mixed gently. Then, the diluted liposomes were added to the diluted DNA and mixed together with vortex. The mixture was held for 20 min at room temperature to enable lipoplexes formation. Lipoplexes solution (prepared as described above) was added slowly to each well in triplicate. They were incubated at 37 °C in a humidified incubator with 5% CO_2 for 5 h, and then cells were washed by PBS and DMEM once. The medium was exchanged with fresh and complete DMEM culture media and cells were further cultured for 48 h, prior to analysis. Finally, the transfection results were observed under the fluorescence microscope equipped with the fluorescent imaging devices for data acquisition and analysis. Transfection efficiency of cationic liposomes was assessed using luciferase. Luciferase activity was normalized by the total protein content and expressed as relative light unit (RLU) per μg of protein (RLU/μg protein).

2.7. Cytotoxicity assay

To determine cell cytotoxicity/viability, cells were plated at a density of 1.0×10^4 cells/well in a 96-well plate at 37 °C under 5% CO_2 atmosphere and incubated 12 h before treatment. The liposome and 50 μL of DMEM medium were added to each well and incubated for 24 h at 37 °C. After incubation, 50 μL of 5 mg/mL MTT solution in PBS was added and incubated for an additional 4 h. MTT-containing medium was aspirated off, and 150 μL of DMSO was added to dissolve the formazan crystal formed by live cells. Cells cultured with DMEM (without liposome) were used as control sample. Cell viability was measured using a microplate reader (BioRAD model 550, Japan) at 550 nm. The relative cell viability related to control wells containing cell culture medium without transfection agents was calculated by $[A]_{test}/[A]_{control} \times 100$.

3. Result and discussion

3.1. Synthesis

Different cationic lipids based on two aliphatic hydrocarbon chains and a quaternary ammonium headgroup, linked by a carbamate bond similar to the cationic lipid N-[1-(2,3-didodecylcarbamoyloxy)propyl]-N-ethyl-N,N-dimethylammonium iodide (DDCEDMA) which has been published, [8] were prepared. As shown in Scheme 1, 1-dimethylamino-2,3-propanediol is synthesized by substitution reaction of dimethylamine and 3-chloro-1,2-propylene glycol, which are readily available from many commercial sources globally. The reaction method has been published by Alquist and Slagh [9] and Liu et al. [10,11], but we find either the reaction requires extreme condition (under high pressure or long reaction time 24 h), or the yield is very low (less than 60%). To solve these problems, we improved the reaction condition by increasing the reaction temperature (50 °C) thereby decreasing the reaction time remarkably (4 h) under normal pressure and increased the net yield (about 70%). We have earlier studied the synthesis of the neutral lipid DDCDMA whose IR, ESI-MS and NMR showed the presence of the expected carbamate-linked and aliphatic hydrocarbon chains (C_{12}) (see ESI). Cationic lipids (CE-DDCDMA, BE-DDCDMA and IE-DDCDMA) bearing the hydroxyl group were synthesized by the same synthetic route as used for preparing cationic lipid DDCEDMA (Scheme 1, see ESI) [8].

3.2. Properties of cationic liposomes

To determine the effect of cationic lipid headgroup structures and cationic lipid to pDNA charge ratio, pDNA complexes were prepared by adjusting the stoichiometry of plasmid and cationic liposomes, using liposomes prepared from a cationic lipid (DDCEDMA, CE-DDCDMA, BE-DDCDMA or IE-DDCDMA) and a co-lipid (DOPE) at identical molar ratios. The electrostatic binding interactions between the plasmid DNA and cationic liposomes at varying lipid/DNA charge ratios were measured by the conventional gel retardation assay.

As shown in Fig. 1, for all cases, the degree of DNA condensation increased with the increase in charge ratio, indicating that these lipids have the ability to form a lipoplex with plasmid DNA. Effective DNA condensation was not achieved with cationic liposomes prepared using DDCEDMA without hydroxyl group at all the charge ratios, as the mobility of pDNA was observed at these charge ratios. In contrast, the mobility of plasmid DNA disappeared above the N/P ratio of 8/1 when the cationic lipid headgroups include