Colloids and Surfaces B: Biointerfaces 145 (2016) 454–461

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Sucrose ester based cationic liposomes as effective non-viral gene vectors for gene delivery

Yinan Zhao[a], Jie Zhu[b], Hengjun Zhou[a], Xin Guo[c], Tian Tian[a], Shaohui Cui[a], Yuhong Zhen[c,*], Shubiao Zhang[a,*], Yuhong Xu[d]

[a] SEAC-ME Key Laboratory of Biotechnology and Bioresources, Dalian Nationalities University, 116600 Dalian, China
[b] The Second Hospital of Dalian Medical University, 116023 Dalian, China
[c] College of Pharmacy, Dalian Medical University, 116044 Dalian, China
[d] College of Pharmacy, Shanghai Jiao Tong University, 200240 Shanghai, China

ARTICLE INFO

Article history:
Received 31 January 2016
Received in revised form 16 April 2016
Accepted 11 May 2016
Available online 12 May 2016

Keywords:
Cationic liposomes
Sucrose esters
Helper lipids
Gene delivery

ABSTRACT

As sucrose esters (SEs) are natural and biodegradable excipients with excellent drug dissolution and drug absorption/permeation in controlled release systems, we firstly incorporated SE into liposomes for gene delivery in this article. A peptide-based lipid (CDO14), Gemini-based quaternary ammonium-based lipid (CTA14), and mono-head quaternary ammonium lipid (CPA14), and SE as helper lipid, were prepared into liposomes which could enhance the interactions between liposomes and pDNA. Most importantly, the liposomes with helper lipid SE showed higher transfection and lower cytotoxicity than those without SE in Hela and A549 cells. It was also found that the transfection efficiency increased with the increase of SE content. The selected liposome, CDO14/SE, was able to deliver siRNA against luciferase for silencing gene in lung tumors of mice, with little *in vivo* toxicity. The results convincingly demonstrated SEs could be highly desirable candidates for gene delivery systems.

1. Introduction

The success of gene therapy mainly depends on the development of efficient and safe vectors for gene delivery. Basically, viral and non-viral vectors have been adapted to introduce genes into cells. Development of non-viral vectors has been one of the primary areas of research because of several significant factors, such as greater carrier capacity, safety, ease of large-scale production, stability, potential to incorporate targeting ligands, and unlimited vector size [1–3]. Among non-viral vectors, cationic liposomes based vectors are widely investigated to improve the transfection efficiency.

Cationic liposomes are composed of lipids, and have improved the gene delivery efficacy owing to their typical bilayer structure. Some helper lipids such as dioleylphosphatidyl choline (DOPC), dioleylphosphatidyl ethanolamine (DOPE), and cholesterol, typically neutrally lipids [4,5], are often employed with cationic lipids. Helper lipids play very important role during the formation of lipoplexes by combining cationic liposomes and genes, as they could determine the morphologies of lipoplexes. Until now SEs are not used to be incorporated into liposomes for this purpose, though they have very broad applications in many fields due to their excellent biocompatibility.

SEs are non-ionic surface-active agents consisting of sucrose as hydrophilic group and fatty chains as lipophilic groups. Through variation of the length or the number of the fatty chains a wide range of hydrophile-lypophile balance values (HLB) can be obtained. In recent years, sucrose esters have attracted much attention because of their biological activities, including insecticidal [6], antitumor [7], and antimicrobial properties [8,9]. SEs have been investigated as promoters in medicine field as a consequence of their surfactant and antimicrobial properties. Owing to their low toxicity, non-antigenicity and biodegradability [10,11], physicochemical properties of these drugs can be tailored to suit potential applications by varying the sucrose head group size and the length and number of alkyl chains, thus can accelerate the speed of drug release, promote dispersion to prevent precipitation, and enhancement of its stability.

In our previous reports [12–14], three types of cationic lipids, Gemini lipids, tripeptide lipids and glyceryl quaternary ammonium lipids were studied to deliver genes. Chemical structures of three cationic lipids are shown in Fig. 1. In this study, we demonstrate the possibility of novel formulations for gene delivery through the lipo-

* Corresponding authors.
E-mail addresses: zhenyuhong@gmail.com (Y. Zhen), zsb@dlnu.edu.cn (S. Zhang).

http://dx.doi.org/10.1016/j.colsurfb.2016.05.033
0927-7765/

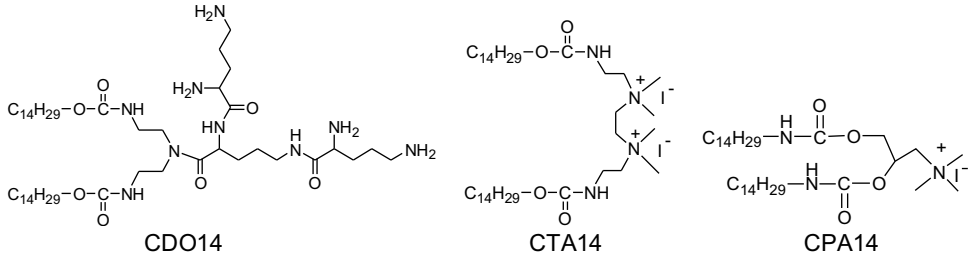

Fig. 1. Chemical structures of cationic lipids. They all have double carbon chains of 14 as tails, among them CDO14, CTA14 and CPA14 have tripeptide, double quaternary ammonium and mono quaternary ammonium as headgroups, respectively.

somes composed of the above cationic lipids and SE as the helper lipid. The transfection activity and safety of liposomes both in vitro and in vivo were investigated. We hope to explore the untouched field by using SEs as helper lipids to extend the cationic liposomes for gene delivery.

In this paper the ratios of lipids to SE and the ratios of liposomes to DNA (RNA) were weight ratios, unless otherwise indicated.

2. Materials and methods

2.1. Materials

Sucrose ester (SE, diester, 18C) was purchased from the Kaiteki company (Japan). Dulbecco's modified Eagle's medium (DMEM), RPMI1640, fetal bovine serum (FBS) and Lipofectamine 2000 were purchased from Invitrogen Life Technologies (USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Gibco (USA). Luciferase siRNA (Luc-siRNA), Sense 5′-CUUACGCUGAGUACUUCGATT-3′, Antisense 3′-TTGAAUGCGACUCA UGAAGCU-5′, Non-targeted siRNA (N-siRNA), Sense 5′-UUCUCCGAACGUGUCACGUTT-3′, Antisense 3′-ACGUGACACGUUCGGAGAATT-5′. Bright-Glo™ Luciferase Assay System was purchased from Promega Biotech Co. Ltd. (Beijing, China). Hela cells were obtained from the Institute of Biochemistry and Cell Biology (China). A549 cells were from Leaf's lab of the University of North Carolina at Chapel Hill. Agarose was purchased from Gene Tech (Shanghai, China). pZsGreen1-N1 plasmid was purchased from Clontech (USA), and extracted in our lab. All other chemicals were of reagent grade. All materials were used as received. All water used was purified using a Milli-Q Plus 185 water purification system (Millipore, USA), giving a resistivity greater than 18 MΩ cm.

2.2. Liposomes preparation and characterisation

The protocol for preparation of liposomes based on CDO14, CTA14 and CPA14 lipids was optimised to obtain high quality delivery systems. The lipids were formulated in combination with the helper lipid SE at lipid/SE (N/S) weight ratios of 1:0–4:1. To prepare liposomes, a suitable amount of lipid and SE were dissolved in 1 mL of chloroform in a glass vial. The solvent was removed under a stream of nitrogen gas, followed by high-vacuum desiccation. The dry lipid film was resuspended in 1 mL distilled water to give liposomes in a concentration of approximately 1 mg/mL. Liposomes suspensions were subjected to several cycles of sonication in a bath sonicator and vigorous vortex mixing to form small vesicles. To prepare lipoplexes, cationic liposomes were mixed with pGL3 DNA in DMEM at liposome/DNA (N/P) weight ratios from 0:1 to 8:1 and incubated for 20 min at room temperature. For the measurement of particle size and Zeta potential, 20 μL of liposomes or lipoplexes were diluted with distilled water (1 mL). Particle size and zeta potential were then measured three times using a Nano-Partikelanalysator (HORIBA SZ-100, Japan).

2.3. DNA-binding assay

Formation of lipoplexes between cationic liposomes and pDNA was confirmed by gel retardation assay. Electrophoresis studies were conducted on 1.2% agarose gels containing 10% GelRed in 1 × TAE buffer. Ten μL of lipoplex was mixed with 2 μL of loading buffer and subjected to agarose gel electrophoresis for 40 min at 80 V. The electrophoresis gel was visualized and digitally photographed using a gel documentation unit (Syngene, Britain).

2.4. In vitro *pDNA transfection*

Hela cells (5.0×10^4 cells/well) were seeded in 24-well plates, and incubated at 37 °C under 5% CO_2 until approximately 80% confluence was attained. To prepare cationic liposome/DNA lipoplexes, plasmid DNA (pZsGreen1-N1) and liposomes were each diluted in 50 μL DMEM; then, the plasmid DNA solution was added to the liposome suspensions. This mixture was incubated at room temperature for 20 min before use to allow the formation of lipoplexes. The medium was removed and replaced with 100 μL serum-free or serum-containing DMEM per well. The lipoplexes were then added to the plates and kept at 37 °C under 5% CO_2 for 4 h. The medium was then replaced with DMEM containing 10% FBS and 1% antibiotics, and the cultures were maintained at 37 °C under 5% CO_2 for 48 h [15]. The expression of green fluorescent protein was measured using an inverted fluorescence microscope (Olympus IX71, Japan) and a flow cytometry (Becton-Dickinson, Heidelberg, Germany).

2.5. In vitro *siRNA transfection*

A549 cells expressing firefly luciferase were seeded in 24-well plates approximately 24 h before experiments. Cells were treated with different lipid formulations at the anti-luciferase siRNA concentration of 40 nM in RPMI1640 at 37 °C for 4 h. The medium was then replaced with RPMI1640 containing 10% FBS and 1% antibiotics, and the cultures were maintained at 37 °C under 5% CO_2 for 48 h. Cells were washed with PBS, followed by incubation with lysis buffer at room temperature for 20 min. Luminescence intensity in cell lysate was determined using a Bright-Glo™ Luciferase Assay System (Promega) and Synergy™ 2 Multi-Detection Microplate Reader (BioTek, USA). Cell lysate (5 μL per well) was dissolved in BCA protein assay reagent, and total protein concentrations were determined at 570 nm. The silencing rate was expressed as relative light units (RLU) per microgram of total protein.

2.6. Evaluation of cytotoxicity of lipoplexes

Cytotoxicity of lipoplexes was determined by MTT reduction assay [16]. The assay was performed in 96-well plates by maintain-