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Rational design of didodecyldimethylammonium bromide-based nanoassemblies for gene delivery

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ABSTRACT

Nonviral gene vectors are a hot topic for gene delivery. High cost and low transfection efficiency hinder the application of them. The aim of this study was to find out the optimal gene vectors with lower cost and more effective gene delivery than commonly used gene vectors. A cheap cationic lipid, didodecyldimethylammonium bromide (DDAB) was the basic component and the other components included oleic acid (OA), cholesterol (Chol), cholesteryl succinyl poly(ethylene glycol) 1500 (CHS-PEG), poly(D,L-lactide-co-glycolide)-methoxy-poly(ethylene glycol) (PLGA-PEG). The combinations of DDAB/OA/Chol, DDAB/OA/CHS-PEG and DDAB/PLGA-PEG were adopted to prepare the nanoassemblies named CNA, CPNA and PPNA, respectively. The optimal component ratios were screened out according to their Langmuir monolayer behavior. The optimal preparation method of nanoassemblies involved firstly compressing DNA or siRNA with the cationic lipid (DDAB) and secondly being coated with the helper lipids (OA and CHS-PEG) or the helper polymer (PLGA-PEG). The complexes of genes and cationic lipids were encapsulated into the core of CPNA and PPNA. The optimal gene vectors (CPNA and PPNA) with small sizes, low negative surface charges and non-exposure of cationic lipids were achieved. They had the advantages of no cytotoxicity, high transfection efficiency and low cost. More importantly, CPNA and PPNA were not sensitive to serum and showed the similar or higher transfection efficiency of pDNA and siRNA compared to Lipofectamine 2000. CPNA could mainly enter cell plasma based on endocytosis. The rational design method is useful for the design and optimization of DDAB-based gene carriers and other cationic lipid-based carriers.

1. Introduction

Gene delivery becomes a key problem with progress of genomics and arising of gene therapy. Current gene delivery techniques are the effective gene vectors that compact and protect oligonucleotides because free oligonucleotides, DNA and siRNA are rapidly degraded in blood. Viruses including retroviruses and adenoviruses are firstly considered as gene vectors. However, toxicity, immunogenicity, and difficult scale-up production limit the clinical application of viral vectors. Nonviral vector systems including cationic lipids, polymers, dendrimers and peptides attract attention due to their biocompatibility and potential of large-scale production. Novel cationic compounds that can form complexes with DNA and can avoid both in vitro and in vivo barriers for gene delivery are focused on [1–3].

Small cationic lipids may be the most promising materials to prepare effective gene vectors [4]. Several cationic lipids have been commercially available, such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl-ammonium chloride (DOTMA), 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), dioctadecylamido-glycylspermine (DOGS). Generally, some helper lipids, such as dioleoylphosphatidylethanolamine (DOPE) and cholesterol, are always used in the formulations for gene delivery. Some popular marketed gene transfection agents also contain these lipids (Table 1). However, these lipids and transfection agents are very expensive, especially for the researchers in developing countries and they are an unavoidable important part of the expenditure of biomedical research.

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Table 1
The formulations of some common marketed transfection agents.[a]

Trademark	Lipid formulation	Company
DMRIE-C®	DMRIE[b]:Cholesterol	Invitrogen
Lipofectin®	DOTMA:DOPE	Invitrogen
Lipofectamine® 2000[c]	DOSPA:DOPE	Invitrogen
Cellfectin®	TM-TPS[d]:DOPE	Invitrogen
Transfectam®	DOGS	Promega
Tfx-50®	Tfx-50[e]:DOPE (1:1)	Promega

[a]The information was from Internet.
[b]DMRIE: 1,2-dimyristyloxy-propyl-3-dimethyl-hydroxy ethyl ammonium bromide.
[c]The price of a vial (1.5 ml) Lipofectamine® 2000 Transfection Reagent at www.lifetechnologies.com was 4500 Chinese Yuan (RMB), or about 528 USD on June 25, 2014. A commonly used cationic lipid in transfection reagents, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium (DOTAP) chloride, is very expensive with the price of 290.50 USD for a 50-mg package at www.sigmaaldrich.com on June 25, 2014. The cationic lipid used in this study, didodecyldimethylammonium bromide (DDAB), is very cheap with the price of 362.50 USD for a 50-g package at www.sigmaaldrich.com on June 25, 2014. The price of DDAB is only about 1/800 of DOTAP.
[d]TM-TPS: N,N^1,N^2,N^3-tetramethyltetrapalmitylspermine.
[e]Tfx-50: N,N,N′,N′-tetramethyl-N,N′-bis(2-hydroxyethyl)-2,3-di(oleoyloxy)-1,4-butanediammonium iodide.

The complex consisting of cationic lipids and gene cargos is called lipoplex. Two fundamental models are used to describe lipoplex structures. One is the "external" model wherein DNA molecules adsorb on the surfaces of cationic liposomes, and the other is the "internal" model wherein DNA molecules are surrounded or coated by a lipid envelope [5,6]. Significantly, the first model is easily prepared although it is highly influenced by the environment and hardly remains in the blood circulation due to its rapid damage. The preparation procedure of the second model is complicated and the entrapment efficiency is not ensured. In addition, high DNA loading efficiency needs the addition of a lot of lipid molecules that would further lead to toxicity due to the cationic property of lipids.

Langmuir monolayers are the regular 2-dimensional monomolecular layers consisting of a lot of amphiphiles at the interface, generally at the air/water interface [7]. Langmuir monolayers are also usually used for investigating amphiphilicity and possible interaction between amphiphiles [8,9]. Some important information about the molecular configurations of amphiphiles can be predicated according to their monolayer behavior. Liposomal formulations can also be investigated with Langmuir monolayers [10]. The information of liposomal components' interaction at the air/water interface may reflect the interaction of them in the lipid vesicles.

A cationic lipid, didodecyldimethylammonium bromide (DDAB) is used as gene delivery vectors to form liposomes [11,12], niosomes [11], and nanoparticles [13,14]. The gene delivery efficiency of DDAB-based vectors is confirmed. Moreover, DDAB is a very cheap reagent (Table 1). In this study, DDAB-based nanoassemblies were explored. The optimal formulations and preparation process were rationally designed according to the Langmuir monolayer behavior of lipids, the stability, entrapment efficiency, physicochemical properties and gene delivery efficiency of nanoassemblies. "Rational design" is emphasized and based on Langmuir monolayers and new gene carrying modes in this study. The rational design method is useful for the design and optimization of DDAB-based gene carriers and other cationic lipid-based carriers, which is thoroughly indicated and confirmed in this study.

2. Materials and methods

2.1. Materials

DDAB was presented by Xiamen Pioneer Technology Inc., China. Oleic acid (OA) was purchased from Tianjin Henghao Kegongmao Co., Ltd., China. Cholesteryl succinyl poly(ethylene glycol) 1500 (CHS-PEG) was synthesized in our lab referred to a previously published protocol [15]. An amphiphilic copolymer, poly(D,L-lactide-co-glycolide)-methoxy-poly(ethylene glycol) (PLGA-PEG) was purchased from Jinan Daigang Biomaterial Co., Ltd., China. The PLGA part had the mean MW of 8000 Da and the lactide/glycotide molar ratio of 50/50. The PEG part had the mean MW of 2000 Da. Organic solvents were of analytical grade and other chemicals were of reagent grade. Purified water was prepared with Heal Force Super NW Water System (Shanghai Canrex Analytic Instrument Co., Ltd., China), and always used. Fluorescein isothiocyanate (FITC) was from Sigma. The pGL3-Basic plasmid expressing *Photinus pyralis* luciferases and the pRL-TK plasmid expressing *Renilla reniformis* luciferases with the cytomegalovirus promoter were supplied by Promega. Plasmids were extracted after transformation using E.Z.N.A. Kit (Omega, USA) and further purified by alcohol precipitation.

2.2. Langmuir monolayer investigation

The surface pressure–molecular area (π–A) isotherms of amphiphilic lipids and polymers were measured on a Minitrough film balance (KSV, Finland) equipped with the dual barriers and a Pt Wilhelmy plate-sensing device. The Teflon trough had a width of 75 mm and an area of 24,300 mm^2. The subphase was purified water (pH 6.0). The experiments were performed at 20 °C. The solutions of DDAB, cholesterol (Chol), OA, CHS-PEG and PLGA-PEG in chloroform were separately prepared. Each solution (different volume from 15 to 50 μl according to the initial surface pressure) was precisely deposited onto the water subphase with a microsyringe. Compression was initiated after a delay of 15 min to allow evaporation of the spreading solvent. The compression rate was 10 mm/min. The mixture solutions of the above components with various molar ratios were also explored as described above.

2.3. Preparation of nanoassemblies

Two preparation methods were used to load genes in this study. One method was based on electrostatic attraction between the cationic nanoassemblies and the anionic genes, like the lipoplexes based on cationic liposomes. The conventional gene-loaded nanoassemblies were abbreviated to CNA. Another method was based on gene compression using cationic lipids to form the micelles that were coated with helper amphiphilic molecules. If the helpers contained CHS-PEG or PLGA-PEG, the nanoassemblies were separately named CPNA or PPNA.

CNA was prepared as the following process. A mixture of the optimal formulation DDAB/OA/Chol (2/3/2, mol/mol) based on the analysis of Langmuir monolayer behavior was dissolved in ethanol and the solution was slowly injected into water. Ethanol was removed under vacuum and the suspension was concentrated to ~7.5 mg DDAB/ml after evaporation. Aliquots of the suspension were gently mixed with a series of DNA solutions to achieve the DDAB/DNA ratios of 1:1, 4:1, 8:1, 16:1 and 64:1 (w/w), respectively. They were incubated at room temperature for 20 min to get CNA. DNA entrapment was measured by a common method of agarose gel electrophoresis.

CPNA and PPNA were prepared using the similar method as described above. A DNA aqueous solution was injected into a vortexed DDAB solution in ethanol with the water/ethanol volume ratio of 1:20. Like CNA, the ratios of DDAB/DNA were also set to 1:1, 4:1, 8:1, 16:1 and 64:1 (w/w), respectively. All of the suspensions were incubated at room temperature for 10 min to make DNA thoroughly complexing with DDAB based on the electrostatic attraction between them. The CHS-PEG/OA mixture or PLGA-PEG was dissolved in ethanol and mixed with the above DNA suspensions. The