The Effect of Lipopolymer Structure on the Transfection Efficiency of Hydrophobic Polyethylenimine-based Cationic Nanoliposomes

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Abstract: In the present study, polyethylenimine (PEI, 1800 Da) and liposome were combined in order to improve gene expression. A new gene delivery system (polycationic liposome) was developed by modification of liposomes with lipoploymers constructed from acrylate or bromoalkane derivatives. The polycationic liposome-plasmid DNA (pDNA) complexes were characterized for their size, zeta potential and ability for DNA condensation. Luciferase reporter gene was used for the determination of transfection efficiency in Neuro2A cells. While mean particle size of prepared vectors ranged from 75 to 520 nm, the zeta potential varied from 11-35 mV. Transfection activity of selected non-viral vectors was higher than that of PEI 1800 Da and PEI 25 KDa. The transfection activity of polycationic liposomes was reduced after replacement of bromoalkane derivatives by acrylates in the structure of lipopolymers. Furthermore, gene carriers described in this study showed low cytotoxicity. The results show that inclusion of hydrophobic PEI derivatives in liposome structure can improve the transfection efficiency but the lipopolymer structure determines the gene expression efficiency of vectors.

Keywords: Lipopolymer, liposomes, non-viral gene delivery, polyethylenimine.

INTRODUCTION

There are two methods for gene transfer, viral and non-viral. Although non-viral carriers are less efficient compared to viral vectors but some advantages such as ease of preparation and safety concerns make them to be considered as favorable alternative to viral vectors [1, 2].

Non-viral vectors protect nucleic acid from degradation and facilitate cellular uptake and trafficking [3, 4]. Several biological barriers such as traversing cell membrane, endosomal membrane and nuclear envelope should be overcome to achieve enhanced transfection [5, 6]. These gene delivery systems are divided into two main groups, polyplexes and lipoplexes. Polyplexes are formed by interaction of cationic polymers and nucleic acid while lipoplexes are composed of cationic lipids and nucleic acid [7]. Recently, polycationic liposomes were developed that composed of two parts, cationic polymer and liposome. These structures were introduced in order to improve gene transfer because of combining the advantages of both lipoplexes and polyplexes [8, 9].

It has been shown that modification of low molecular weight polyethylenimines (PEIs) by hydrophobic moieties enhanced transfection activity while transfection efficiency was reduced by hydrophobic modification in high molecular weight PEIs [9]. Also, inclusion of low molecular weight PEIs (MW 700 and 2000 Da) into liposome structure was evaluated by Lampela *et al.* They showed the synergistic increase in transfection efficiency by this combination [10]. To improve the target specificity and biological activity of short synthetic oligodeoxynucleotides, three different lengths of poly(l-lysine) were conjugated to oligodeoxynucleotide and these conjugates were encapsulated in *N*-stearyllactobionamide-modified liposomes. The results showed that the modified liposomes and poly(l-lysine) modification increased uptake of oligodeoxynucleotide by HepG2 cells [11]. Polyamine conjugates of dialkyl phosphates, combined with natural lipids and assembled in the form of liposomes were prepared in the study of Dewa *et al.* [12]. They described that selected polycationic liposomes exhibited 3.6 times higher activity than that of a popular commercial product.

Previously, we showed that small and large molecular weight linear PEI in combination with liposome could improve gene expression [13]. However, in the present study covalent modification of liposomes with hydrophobically modified low molecular PEI was evaluated.

MATERIALS AND METHODS

Materials

1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) were purchased from Avanti Polar Lipids (Albaster, AL, USA). Different branch PEIs including 1800 Da, 10 KDa and 25 KDa were obtained from Polysciences, Inc. (Warrington, PA, USA). Bromohexane, bromodecane and bromooctane from Aldrich (Germany) were used as bromoalkane derivatives. Acrylate derivatives including hexyl acrylate, isodecyl acrylate and octadecyl acrylate were from Aldrich (Germany). All other materials were of analytical grade.

Synthesis of Lipopolymers

PEI 1800Da was dissolved in chloroform. The desired amount of acrylate or bromoalkane derivative was added to the solution with stirring. The reaction proceeded at 45-50 °C. After 4 hour, the organic solvent was removed and the product was freeze-dried. The synthesized lipopolymer was then characterized by FT-IR, TLC and ^{1}H-NMR [13].

Preparation of Polycationic Liposomes

A mixture of lipid and lipopolymer at molar ratio of 1:1 was dissolved in chloroform: methanol (2:1, v/v) in a round-bottom flask. The solvent was removed by rotary evaporator (Heidolph, Schwabach, Germany) resulting in the deposition of a thin lipid film on the flask wall. For complete removal of organic solvents, this lipid film was freeze-dried (Heto Drywinner, Birkerod, Denmark) overnight. The lipid film was then hydrated while vortexing. The size of liposomes was reduced by thermobarrel Extruder (Northernlipids, Vancouver, Canada). The polycationic liposomes

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