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Characterization and in vivo performance of dextran–spermine polyplexes and DOTAP/cholesterol lipoplexes administered locally and systemically

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Abstract

In this study, we compared two systems which can be applied for transfection in vitro and in vivo: polyplexes based on the polymer dextran–spermine (D–SPM) and lipoplexes based on 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)/cholesterol. The carriers differ in (1) solubility in aqueous media, (2) source of the positive charges (quaternary amines for DOTAP and primary plus secondary amines for D–SPM), (3) electrostatics, i.e., for lipid and polymer, respectively: zeta-potential (81.0 and 48.1 mV), surface potential (180 and 92 mV), and surface pH (10.47 and 8.97), and (4) charge distribution (ordered versus non-ordered). The stability of the complex upon interaction with serum proteins was studied by means of fluorescence resonance energy transfer (FRET) between rhodamine-labeled cationic carriers and fluorescein-labeled DNA. Addition of serum increases the lipid–DNA average distance and decreases the polymer–DNA distance. However, FRET efficiency indicates that serum proteins do not induce a major DNA dissociation for either polyplexes or lipoplexes. Comparing the biodistribution of rhodamine-labeled complexes and the transgene expression after intravenous (i.v.), intramuscular (i.m.), and intranasal (i.n.) administration, we found that local administration of lipoplexes resulted in the lipoplexes remaining at the site of injection, whereas the polyplexes showed systemic distribution, accompanied by transgene expression in lungs and liver. It is suggested that the high water-solubility of the polymer combined with its lower positive charge (compared to DOTAP), which makes its association with the cells at the site of injection weaker, enables the polymer to reach and transfect distant organs through the blood stream. Using chemically modified D–SPM, we demonstrated the importance of high density of positive charges and a sufficient level of secondary amines for achieving efficient transgene expression in vivo.

Keywords: Gene delivery; Charge-distribution; PEGylation; Biodistribution; Transgene expression

1. Introduction

Gene delivery using non-viral assemblies, especially the self-assembled complexes between negatively charged nucleic acids and cationic carriers (lipids or polymers), has gained increased interest as an alternative for viral vectors. The attractiveness of such complexes is a result of several advantages over viral vectors, especially their superior safety profile [1]. However, these non-viral gene delivery systems have several major drawbacks including lack of specificity, cell toxicity, low biodegradability, stability, and especially, low transfection efficiency. In addition, their rapid clearance from the blood circulation limits potential transfection sites to first-pass organs, especially the lungs [2–4].

There are several possible mechanisms that might contribute to the rapid blood clearance of complexes. Large and hydrophobic particles interact with and are taken up faster by cells of the reticuloendothelial system (RES) [5]. Particles with strong positive surface charge bind

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to negatively charged biological membranes nonspecifically. In addition, the blood contains proteins and glycoproteins that can function as opsonins [6]. Albumin is the most common negatively charged plasma protein. Therefore, it may be the first component to be adsorbed to the surface of positively charged complexes, forming ternary hydrophobic complexes and excessive aggregation [7]. Also, filtering by capillary beds was proposed as a possible mechanism that might contribute to the rapid clearance of complexes from the blood.

Attachment of hydrophilic polymers such as polyethyleneglycol (PEG) and poly(*N*-(2-hydroxypropyl)methacrylamide) (pHPMA) at the positively charged particle surface was proposed to reduce interactions with proteins and cells through a mechanism referred to as steric stabilization [5,8–10]. However, despite showing promising results in vitro, incorporation of shielding molecules during the initial self-assembly with DNA usually did not result in better distribution kinetics in vivo [11,12], while compromising uptake by the cells [13]. It was suggested that the introduction of lateral stabilization, using multivalent coating polymers, is needed in order to provide better stability of polyplex particles than simple steric stabilization [10,12].

In spite of the extensive information gained on lipoplexes and polyplexes in the last few years, there are still many gaps when relating their physicochemical properties to their transfection efficiency and toxicity. One of the best ways to fill in these gaps is to compare well-characterized representatives of these two carrier systems that are efficacious in vitro and in vivo, and to correlate physicochemical features with their in vivo performance. This study was dedicated to meet this objective. We compared lipoplexes based on the mixture of the monocationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and the neutral helper lipid cholesterol [4], and our novel (but well characterized) polyplexes based on the cationic polymer dextran–spermine (D–SPM) [14–18]. Although these two systems have some similarities, such as being able to self-assemble with DNA and form large assemblies upon interaction with negatively charged serum proteins, they differ to a large extent in most of their other physicochemical properties. We demonstrate that these differences between the two systems are responsible for their different performance in vivo. First we compared the physicochemical properties of these two systems step by step (size distribution in buffer solution and in "high" (200 μl/ml) and "low" (20 μl/ml) serum concentrations, solubility in aqueous media, the effect of serum on DNA–cationic carrier average distance, zeta-potential, surface potential and surface pH). Then we correlated all the above properties with complex biodistribution and transgene expression using the intravenous (i.v.), intranasal (i.n.) and intramuscular (i.m.) routes of administration. In order to understand how polymer steric stabilization, positive charge and its distribution on the polymer backbone affect transgene expression, we evaluated transfection efficiency of D–SPM derivatives including PEGylated D–SPM (5% of the ε-NH_2 were PEGylated with PEG 2000 Da) and "low-sperminated" D–SPM (in which the amount and the distribution of cationic charge on the polymer backbone is lower compared to unmodified D–SPM).

2. Experimental materials and methods

2.1. Materials

DOTAP and lissamine rhodamine-phosphoethanolamine (LRPE) were obtained from Avanti Polar Lipids (Alabaster, AL), cholesterol and dextran (40 kDa) from Sigma (St. Louis, MO), 7-hydroxycoumarin-3-carboxylic acid-succinimidyl ester, Rhodamine-B, and fluorescein isothiocyanate (FITC) from Molecular Probes (Eugene, OR), and spermine from Fluka (Buchs, Switzerland), The pH-sensitive probe 7-hydroxycoumarin-phosphoethanolamine (HCPE) was prepared in our laboratory [3].

2.2. Mice

Specific pathogen-free (SPF) female BALB/c mice, 6–8 weeks old, were used. Animals were maintained under SPF conditions. The Institutional Animal Care and Use Committee approved all the procedures used in this study, consistent with the guide for the care and use of laboratory animals.

2.3. Preparation of dextran–spermine conjugates

The synthesis and the chemical characterization of D–SPM and low sperminated D–SPM including the determination of primary amines by the trinitrobenzenesulfonic acid (TNBS) method were detailed in our previous publications [14–16]. The synthesis of 5% PEGylated D–SPM from methoxy-PEG_{2000}-terminated *p*-nitrophenyl carbonate (mPEG-OPNC) was detailed in our previous publication [19]. Hydroxycoumarin-labeled polymer was prepared as follows: D–SPM was dissolved under gentle stirring in water. A solution of 7-hydroxycoumarin-3-carboxylic acid-succinimidyl ester in dimethyl formamide (DMF) was added at 0.75 mol% relative to polymer ε-NH_2. After stirring in the dark at RT for 24 h, the HC-labeled polycations were purified by Sephadex G-10 (Amersham Pharmacia Biotech, Sweden) column chromatograpy using water as eluent. Fractions containing the labeled polycation were collected and lyophilized to dryness. D–SPM labeled with Rhodamine-B was prepared similarly by reaction of the polymer with Rhodamine-B sulfonyl chloride (0.5%).

2.4. Liposome preparation

Unsized heterogeneous vesicles (UHV) were prepared by overnight lyophilization of a mixture of DOTAP and Cholesterol (1:1 mol/mol) dissolved in *tert*-butanol. The lyophilized cake was hydrated with 20 mM Hepes, pH 7.4, and vortexed for several minutes to form UHV (~500 nm), which are mostly uni- and oligolamellar vesicles [3]. Labeled liposomes were prepared by adding HCPE or LRPE to the mixture of DOTAP and Cholesterol (1:1 mol/mol) dissolved in *tert*-butanol at 0.5 (HCPE) and 1 (LRPE) mol% relative to DOTAP.

2.5. DNA preparation

*p*LNCluc *p*CMVLacZ plasmids were prepared from bacterial culture, and purified using Qiagen Plasmid Mega Kit (Hilden, Germany). The concentration of DNA was quantified by determination of organic phosphorus [20], which represents DNA negative charges. Fluorescein-labeled DNA was a generous gift of D. Simberg.