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Biodistribution and tissue expression kinetics of plasmid DNA complexed with polyethylenimines of different molecular weight and structure

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Abstract

Polyethylenimine (PEI) has been studied as an efficient and versatile *in vitro* and *in vivo* gene delivery agent. Here, we report the *in vivo* fate, tissue expression duration, and safety after the intravenous injection of plasmid DNA complexed to various PEIs under different conditions. Murine interleukin-2 plasmid DNA was complexed with branched PEI2Kd, 25Kd, or linear PEI25Kd at different *N*/*P* ratios. The mean residence time of plasmid DNA was found to be prolonged after delivery in PEI, evidencing the highest values in branched PEI25Kd. As compared to branched PEI25Kd, linear PEI25Kd at the same *N*/*P* ratio provided mRNA expression levels orders of magnitude higher in the lung over an 8-day period. In the branched PEI2Kd/DNA complexes, the *N*/*P* ratio of 80:1 evidenced higher gene expression efficiency in the kidney and spleen than the normal *N*/*P* ratio of 10:1. The generation of proinflammatory chemokine receptors was induced by branched PEI25Kd, but not by other PEIs. The complexes of DNA with linear 25Kd PEI or branched PEI2Kd exhibited no histological changes after repeated administrations. These results indicate that the structure, molecular weight, and *N*/*P* ratios of PEIs must be collectively considered and modulated for organ-targeted plasmid DNA delivery.

Keywords: Polyethylenimine; Nonviral gene delivery; Biodistribution; Low molecular weight; Safety

1. Introduction

Currently available gene delivery systems can be classified into viral and nonviral systems [1]. Generally, viral vectors have been shown to result in significantly higher levels of gene expression than were seen with nonviral vectors [2,3]. Despite such impressive gene transfer statistics, the unresolved chief concern remains the toxicity of the viruses and the potential for generating a strong immune response, owing to the relevant capsid proteins [4]. Meanwhile, the use of nonviral delivery systems has been theorized to circumvent some of the problems associated with viral vectors, and combine lack of immune response with ease of formulation [5,6].

Among the known cationic nonviral vectors, polyethylenimine (PEI) has been evaluated with regard to its efficacy as a versatile, inexpensive, and useful transfection system [7]. *In vivo*, PEI has been demonstrated to be an efficient transfection vector in several organs, including the liver and lung, and evidences prolonged organ retention kinetics [8]. Until now, in most studies involving PEI, the most frequently-utilized PEIs were sized in a range between 22–25Kd, and had a branched or linear structure. With regard to the effects of the structure of PEI, recent studies have reported that branched and linear PEI (m.w. 22–25Kd) may differ in terms of the transfection efficiency of genes in some cell lines, or following aerosol delivery [9,10]. In terms of size, lower molecular weight PEI2Kd (m.w. 2Kd) was

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demonstrated to be safer and more effective for gene delivery into hematopoietic progenitor cells, including human CD34 and murine Sca I cells, than was PEI25Kd (m.w. 25Kd) [11]. A low molecular weight fraction of polyethylenimine (PEI) has been reported to display increased DNA transfection efficiency in carcinoma cells [12]. Moreover, gene expression efficiency was determined to be affected significantly by the PEI nitrogen to DNA phosphate (*N*/*P*) ratios between PEI and plasmid DNA in several cell lines [13,14]. However, little knowledge has been gathered thus far regarding the effects of molecular weights and types of PEI on the *in vivo* fate and expression levels of plasmid DNA after systemic delivery.

In this study, we aimed to increase the understanding of the in vivo fate of plasmid DNA delivered by various PEIs. Here, we have assessed the biodistribution, organ expression kinetics, and proinflammatory chemokine receptor induction, in several organs after gene delivery using the branched or linear forms of differently-sized PEIs.

2. Methods and materials

2.1. Plasmid DNA

Plasmid DNA encoding for murine interleukin-2 (mIL-2) under the control of the cytomegalovirus promoter (pVAXmIL-2) was constructed via the insertion of the 529 bp mIL-2 gene from pCIneoIL-2 into the pVAX1 expression vector (Invitrogen, Carlsbad, CA, USA) using *Nhe*I and *Bam*HI restriction enzymes [14]. The competitor internal standard, pVAXdmIL-2, a 173-bp deleted mutant of pVAXmIL-2, was constructed via the insertion of the 356-bp partial fragment of the mIL-2 gene from pCIneoIL-2 into the pVAX1 vector, using the *Nhe*I and *Hin*dIII sites [14]. The plasmid DNA was amplified in *Escherichia coli* DH5α, and purified with a Qiagen Giga prep kit (Qiagen, Valencia, CA, USA). The purity of the DNA preparations was confirmed via 1% agarose gel electrophoresis.

2.2. Formation of PEI/DNA complexes and size measurement

PEI/mIL-2 complexes of various *N*/*P* ratios were prepared via the addition of the appropriate amounts of PEI (branched 2Kd and 25Kd, Sigma Aldrich, Saint Louis, MO, USA; linear 25Kd, Polyscience, Warrington, PA, USA) to mIL-2 plasmid DNA. The PEI stock solutions were prepared at 4.3 mg/ml in distilled water [15]. The solution was slightly acidified to a pH of 5.0 using HCl, and filtered with a 0.2 μm polycarbonate membrane. The *N*/*P* ratios, the ratio of nitrogen of PEI per phosphate residue of plasmid DNA, were calculated based on the amount of PEI nitrogen per DNA phosphate (1 μg of DNA is 3 nmol of phosphate, and 1 μl of PEI stock solution contains 10 nmol of amine nitrogen) [15]. For complexation, the plasmid DNA was diluted to 150 mM NaCl to a final concentration of 10 μg/ml, and PEI stock solution was added at differing volumes. The mixture (100 μl) was then immediately vortexed for 10 s, and incubated for 30 min at room temperature.

The particle sizes of the PEI/DNA complexes were determined with a ELS-8000 dynamic laser light scattering system (Photal, Osaka, Japan). The hydrodynamic diameters of the particles were determined via dynamic He–Ne laser (10 mW) light scattering. Data analysis was conducted using a software package (ELS-8000 software) supplied by the manufacturer.

2.3. Preparation of biological samples

ICR mice were used at 5–6 weeks of age. Female ICR mice were used for most of the experiments conducted in this study. Male ICR mice were employed in order to determine the distribution and expression of plasmid DNA in the testes. Animals received food and water *ad libitum*. Plasmid DNA (50 μg) in naked form or PEI complexes was administered intravenously into the tail veins of the mice using a 30-gauge insulin syringe. At various time points after the administration of plasmid DNA, approximately 50 μl of blood was collected from the tail vein of each mouse, using a 1.1-mm diameter capillary tube. Total serum DNA was extracted using DNAzol reagent (Invitrogen, Carlsbad, CA, USA), in accordance with the manufacturer's instructions. For the biodistribution study, the mice were sacrificed and their organs were harvested. In an attempt to minimize the influence of plasmid DNA in blood circulating through the tissues at the time of sampling, the samples were washed thoroughly several times with saline, then blotted dry and weighed. The samples were then suspended in DNAzol® (Gibco BRL, NY, USA) at a concentration of 50 mg of tissue per ml, and homogenized using an IKA Ultra-Turrax T8 homogenizer (Germany). The homogenates (500 μl) were then loaded onto Wizard® DNA clean up columns (Promega, WI, USA). After washing, the DNA was eluted with 50 μl of TE buffer.

2.4. Quantitative and competitive polymerase chain reaction (PCR)

Quantitative and competitive polymerase chain reaction (PCR) was employed in order to determine the levels of plasmid DNA in the biological samples. Competitive PCR was conducted via the addition of various amounts of the internal standard competitor, pVAXdmIL-2, to reaction mixtures that contained 1 μl of sample DNA of unknown concentration. The primers utilized for competitive PCR were as previously described [14]. PCR was conducted in a final volume of 50 μl containing 1 μl of sample DNA, 1 μl of the competitor plasmid, 4 μl of 2.5 mM dNTPs, 5 μl of 10× reaction buffer (50 mM Tris pH 8.0, 100 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol, and 1% Triton X-100), 10 pmol of each primer, and 1 U of Taq polymerase. PCR amplification was conducted by heating the mixture at 94 °C for 5 min, followed by 33 cycles at 95 °C for 40 s, 56 °C for 30 s, and 72 °C for 30 s, and then at 72 °C for 10 min. The PCR products were separated on 2% agarose gel. The density of each band was measured with a Gel-doc image analyzer (Vilber Lourmat, France), and calibration curves were utilized to quantify the target DNA in the samples. In each competitive PCR run, an internal standard curve was obtained via the plotting of the log values of target/competitor