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GENE DELIVERY

Galactosylated polyethylenimine-*graft*-poly(vinyl pyrrolidone) as a hepatocyte-targeting gene carrier

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Abstract

Polyethylenimine (PEI) has been used for the gene delivery system in vitro and in vivo since it has high transfection efficiency owing to proton buffer capacity. However, the use of PEI for gene delivery is limited due to cytotoxicity, non-specificity and unnecessary interaction with serum components. To overcome cytotoxicity and non-specificity, PEI was coupled with poly(vinyl pyrrolidone) (PVP) as the hydrophilic group to reduce cytotoxicity and lactose bearing galactose group for hepatocyte targeting. The galactosylated-PEI-*graft*-PVP (GPP) was complexed with DNA, and GPP/DNA complexes were characterized. GPP showed good DNA binding ability, high protection of DNA from nuclease attack. The sizes of DNA complexes show tendency to decrease with an increase of charge ratio and had a minimum value around 59 nm at the charge ratio of 40 for the GPP-1/DNA complex (PVP content: 4.1 mol%). The GPP showed low cytotoxicity. And GPP/DNA complexes were mediated by asialoglycoprotein receptors (ASGP-R)-mediated endocytosis. Also, the transfection efficiency of GPP-1/DNA complex at charge ratio of 40 in the HepG2 was higher than that of PEI/DNA one.

Keywords: Gene carrier; Polyethylenimine; Galactose; Poly (vinyl pyrrolidone); Asialoglycoprotein-receptor

1. Introduction

Attention has been mainly focused on developing efficient and adequate gene delivery systems since gene delivery has possible applications in gene therapy for various human diseases such as genetic disorders and cancer. Although viral systems are potentially very efficient, the fundamental limitations of viral-mediated delivery such as toxicity, restricted targeting of specific cell types, limited DNA carrying capacity, production and packaging problems, potential virus recombination, and high cost hinder practical

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application [1,2]. Therefore, the use of non-viral gene carriers such as cationic polymers or cationic lipids may overcome the current problems associated with viral gene carrier in safety, immunogenicity, mutagenesis and cost [3].

Polyethylenimine (PEI) has successfully been used for gene delivery both under in vitro and in vivo conditions. PEI offers a high positive charge density and exhibits a strong proton buffer capacity over a broad pH range. The latter characteristic is advantageous for PEI since the proton sponge effect allows sufficient gene transfer without endosome disruptive reagents. However, the drawbacks such as cytotoxicity and unnecessary interaction with serum components still remains to be overcome for gene delivery in vivo [4,5].

PEI mediated transfection of cells under in vitro conditions is most efficient at an excess of cationic polymer leading to positively charged polyplexes which bind to anionic cell surfaces. This transfection method is quite efficient under in vitro conditions but uptake into cells occurs by adsorptive endocytosis and is non-specific. Thus, more specific methods of polyplex targeting are required for in vivo applications where gene delivery to defined target tissues is clearly desirable. Different strategies for targeted gene transfer using peptides [6,7], proteins [8], transferrin [9,10], folic acid [11,12], or antibodies [13] as homing devices have been studied.

Targeting to liver parenchymal cells is feasible due to the abundance of the asialoglycoprotein receptors (ASGP-R) which is specific for hepatocytes. Naturally, ASGP-R recognizes and internalizes glycoproteins bearing terminal galactose or *N*-acetylglucosamine residues via clathrin-coated pits [14].

Therefore, the combination of polymeric gene carriers with ligands such as galactose [15–19], lactose [20–22], and apolipoprotein E [23] has been studied to deliver genes into hepatocytes. Even though 5% galactose directly conjugated to PEI gave high transfection in hepatocyte-derived cell lines [16], it was not possible to prevent aggregation under physiological saline conditions [18].

The amphipathic poly(vinyl pyrrolidone) (PVP) used in pharmaceutical areas is a synthetic polymer with same properties to poly(ethylene glycol) (PEG), i.e. it is water soluble, non-charged and nontoxic, and is often used as a medicinal additive or polymeric modifier of bioactive proteins [24,25]. The PVP was found to be retained in blood better than PEG. PVP-conjugated TNFα had more than 200 fold and 5 fold higher antitumor efficacy than native TNFα and PEG-conjugated TNFα, respectively [24]. Moreover, PVP has been shown to stabilize protein drug and induce a macromolecular crowding effect on *Escherichia coli* DNA [26,27].

In this study, PEI was used as a gene carrier and conjugated with lactose bearing galactose group for hepatocyte targeting and grafted with PVP as a hydrophilic group to reduce cytotoxicity and to prevent aggregation under physiological saline conditions of PEI.

2. Material and methods

2.1. Materials

Branched PEI 25 kDa, β-lactose, *N*-vinyl pyrrolidone, 2.2′-azobisisobutyronitrile (AIBN), 3-mercaptopropionic acid, sodium cyanoborohydride, and DNA (sodium salt, from calf thymus) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 5.3-kilobase pair expression vector pGL3-control (Promega, Madison, USA) contains luciferase gene driven by SV40 promoter and enhancer. The plasmids were propagated in JM-109 competent cells, *Escherichia coli*, and purified by chromatography (Megaprep Kits, Qiagen, Chatsworth, CA, USA). The purity and concentration of obtained DNA were measured by UV absorbance at 260 and 280 nm. DNA from calf thymus was used for measurement of sizes and zeta potential.

2.2. Synthesis of galactosylated PEI (GP)

GP was synthesized by reductive amination reaction [28,29]. Briefly, 500 mg PEI 25 kDa and 99.5 mg of lactose (10 mol% to primary amine residues of PEI) were dissolved in 0.2 M borate buffer solution and the pH was adjusted to 8.2, followed by the addition of sodium cyanoborohydride (5-fold molar excess of lactose). Then, the reaction mixture was stirred at room temperature for 24 h. It was purified using Spectra/Por membrane (MWCO = 12,000–14,000) and lyophilized. The con-