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A novel reduction-sensitive modified polyethylenimine oligonucleotide vector

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ABSTRACT

A reduction-sensitive cross-linked polyethylenimine derivative PEI-SS-OA was synthesized and evaluated for oligonucleotide delivery. PEI-SS-OA was shown to condense LOR-2501, an oligonucleotide targeting ribonucleotide reductase R1 subunit (RRM1), into positively charged complexes. The reductive degradation of the PEI-SS-OA induced by dithiothreitol was confirmed by a gel retardation assay. In vitro experiments revealed that the reduction-sensitive PEI-SS-OA was less cytotoxic and more effective in oligonucleotide delivery than the control 25 kDa PEI. This study demonstrates that a reducibly degradable cationic polymer PEI-SS-OA possesses both higher oligonucleotide delivery efficiency and lower cytotoxicity than PEI (25 kDa), therefore is an attractive candidate for further in vivo evaluation.

1. Introduction

The ability to mediate high levels of oligonucleotide delivery with low toxicity is one of the most important requirements for clinical application (Huang et al., 2008; Mintzer and Simanek, 2009). Existing vectors have limited efficiency and high toxicity (Bansal et al., 2014; Fabregas et al., 2014; Xu et al., 2014; Yang et al., 2014). Therefore, there continues to be the need to further develop new delivery system with greater efficiency and lower toxicity.

LOR-2501 is a 20-mer phosphorothioate oligonucleotides, which targets ribonucleotide reductase R1 mRNA. Ribonucleotide reductase is an enzyme associated with drug resistance, so LOR-2501 has shown potent antitumor activities (Lee et al., 2006; Nocentini, 1996). The efficacy of LOR-2501 is dependent on its efficient delivery to the cytoplasm. A novel and high-efficiency vector is needed. Polyethylenimine (PEI) is a commonly used cationic polymer for nucleic acid delivery because of its high positive charge density and endosomolytic activity. PEI has strong escape capacity from endosomes due to its unique "proton sponge" effect (Akinc et al., 2005; Benjaminsen et al., 2013; Richard et al., 2013; Rudolph et al., 2005; Q.L. Xie et al., 2013; J. Xie et al., 2013). High molecular weight PEI (25 kDa) has frequently been used but limited due to its cytotoxicity. Previous studies have shown that hydrophobically modification of PEI with a lipophilic moiety greatly improves its transfection activity and reduces its toxicity (Alshamsan et al., 2009; Moghimi et al., 2005; Park et al., 2010; Q.L. Xie et al., 2013; J. Xie et al., 2013).

In recent years, intracellular environment-sensitive systems that release drugs in response to endosomal pH and cytoplasmic glutathione (GSH), have been designed and explored for enhanced cancer therapy (Bae and Kataoka, 2009; Chen et al., 2013; Deng et al., 2012; Peng et al., 2008). Disulfide linkages have shown to be stable in blood circulation and undergo rapid degradation in the presence of reductive 1,4-dithio-DL-threitol (DTT) or glutathione (GSH) which mimics the reductive intracellular environment (Meng et al., 2009; Zhang et al., 2012). Liu et al. synthesized a novel reducible disulfide-containing cross-linked polyethylenimines (PEI-SS-CLs) via click chemistry and evaluated as nonviral gene delivery vectors. The study demonstrated that a reducibly degradable cationic polymer composed of LMW PEI cross-linked via a disulfide-containing linker possesses both higher gene transfection efficiency and lower cytotoxicity than PEI (25 kDa) (Liu et al., 2010). Choi and Lee, conjugated the endosomolytic protein listeriolysin O (LLO) from the intracellular pathogen Listeria monocytogenes with polyethylenimine (PEI) of average molecular weight 25 kDa (PEI25K) via a reversible disulfide bond (LLO-s-s-PEI), results showed that the use of the synthesized LLO-s-s-PEI conjugate, further enhanced the transfection efficiency beyond that of DNA condensates with disulfide-crosslinked PEI (Choi and Lee, 2008).

In this study, a novel reduction-sensitive polymer PEI-SS-OA was synthesized and evaluated as a carrier for LOR-2501. First, PEI

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(25 kDa) was modified with SPDP. Then, this PEI-PDP was reacted with sulfydryl-containing derivative of oleylamine to obtain a reducible PEI derivative PEI-SS-OA. This paper describes the synthesis of a reduction-sensitive polymer PEI-SS-OA and the oligo delivery activities of the corresponding micellar particles.

2. Materials and methods

2.1. Materials

Branched PEI (25 kDa) and Traut's Reagent (2-iminothiolane·HCl) were obtained from Sigma–Aldrich (St. Louis, MO). SPDP was purchased from Dojindo (Dojindo Laboratory, Kumamoto, Japan). Oleylamine was purchased from Xiya Reagent (Xiya Reagent Co., Ltd., China). 3-(4,5-Dimethylthi-azol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma–Aldrich (St. Louis, MO). Fetal bovine serum (FBS) was obtained from Gibco (Gibco BRL Co., Ltd., USA). 4′,6-Diamidino-2-phenylindole (DAPI) was purchased from Invitrogen Molecular Probes (Oregon). LOR-2501 (5′-CTC TAG CGT CTT AAA GCC GA-3') and 5′-Cy3-LOR-2501 were obtained from Biomics Biotechnologies.

2.2. Cell culture

HeLa cells were grown and propagated in Dulbecco's modified Eagle's medium (DMEM), A549 cells were grown and propagated in RPM1640, supplemented with 10% FBS and 1% antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin, Sigma). Cells were grown at 37 °C in humidified air containing 5% CO_2.

2.3. Synthesis of PEI-SS-OA

PEI-SS-OA was prepared by coupling reaction between PEI-PDP and OA-SH (Fig. 1). Briefly, PEI 25 kDa (20 mg, 0.8 μmol) was dissolved into ethanol to achieve concentration 20 mg/ml. Twenty μmol SPDP was dissolved into ethanol to achieve concentration 100 mg/ml. The SPDP solution was added to the polymer solution rapidly under stirring and reacted for 4 h at room temperature to prepare the PEI-PDP. Oleylamine (8 mg, 30 μmol) was dissolved into 0.5 ml ethanol, and 8.25 mg Traut's Reagent (2-Iminothiolane·HCl) was dissolved into water. The Traut's Reagent solution was added to the oleylamine solution under stirring and reacted for 3 h at room temperature to prepare the OA-SH. Next, the OA-SH solution was added to the PEI-PDP solution dropwise. The reaction mixture was stirred at room temperature for 4 h. Finally, the product was dried under nitrogen. The composition of the reaction products was determined by a 300 MHz 1 H NMR spectroscope (Bruker 300 AM; Billerica, MA). The proton shifts specific were integrated, normalized for the number of protons in each peak, and used to obtain the lipid substitutions on polymers.

2.4. Buffer capacity determination of polymers

The ability of polymers to buffer at pH from 10 to 2 was determined by acid–base titration as described previous (Wang et al., 2006). For PEI and PEI-SS-OA, 0.5 mg/ml solution was prepared, the pH was raised to 10 using 1 mol/l NaOH. Increasing volumes of 0.1 mol/l HCl (with 10 μl increments) was added into 3 ml polymer solution and the pH value was measured at the same time using a pH-meter. The experiment was done at room temperature. The pH values were obtained for each polymer and graphs of the data were generated accordingly.

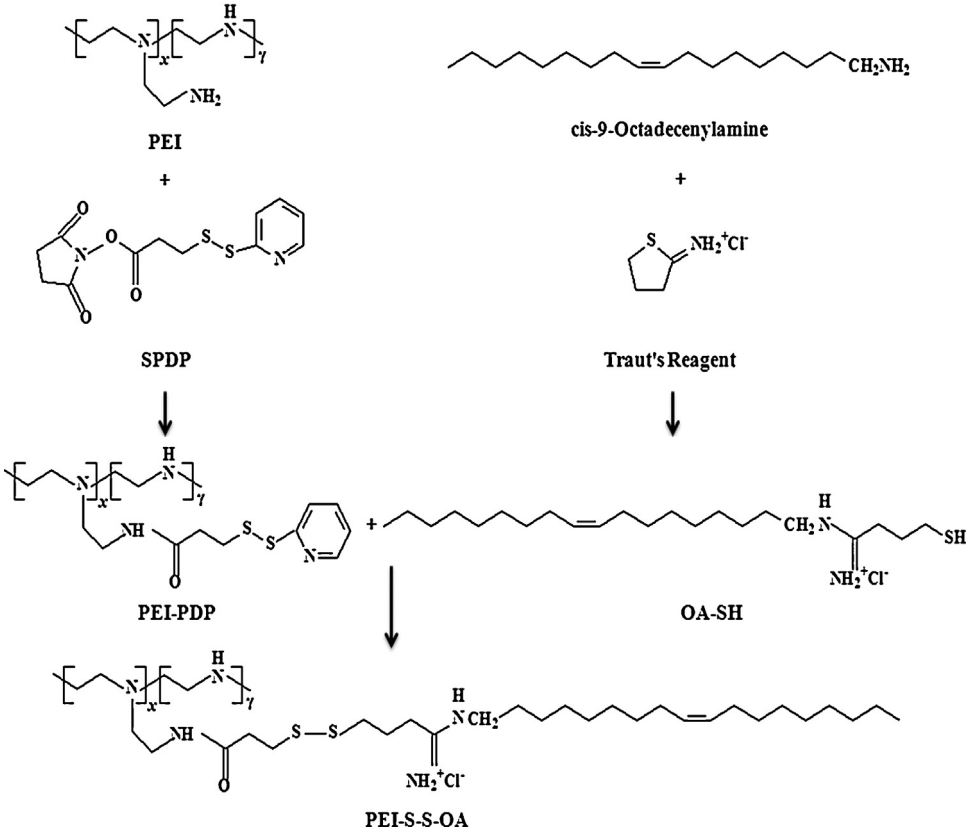

Fig. 1. The scheme of PEI-SS-OA synthesis.