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Biodegradable calcium phosphate nanoparticle with lipid coating for systemic siRNA delivery

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ABSTRACT

A lipid coated calcium phosphate (LCP) nanoparticle (NP) formulation was developed for efficient delivery of small interfering RNA (siRNA) to a xenograft tumor model by intravenous administration. Based on the previous formulation, liposome-polycation-DNA (LPD), which was a DNA–protamine complex wrapped by cationic liposome followed by post-insertion of PEG, LCP was similar to LPD NP except that the core was replaced by a biodegradable nano-sized calcium phosphate precipitate prepared by using water-in-oil micro-emulsions in which siRNA was entrapped. We hypothesized that after entering the cells, LCP would de-assemble at low pH in the endosome, which would cause endosome swelling and bursting to release the entrapped siRNA. Such a mechanism was demonstrated by the increase of intracellular Ca^{2+} concentration as shown by using a calcium specific dye Fura-2. The LCP NP was further modified by post-insertion of polyethylene glycol (PEG) with or without anisamide, a sigma-1 receptor ligand for systemic administration. Luciferase siRNA was used to evaluate the gene silencing effect in H-460 cells which were stably transduced with a luciferase gene. The anisamide modified LCP NP silenced about 70% and 50% of luciferase activity for the tumor cells in culture and those grown in a xenograft model, respectively. The untargeted NP showed a very low silencing effect. The new formulation improved the *in vitro* silencing effect 3–4 folds compared to the previous LPD formulation, but had a negligible immunotoxicity.

1. Introduction

Non-viral vector for the delivery of nucleic acid has been studied since the 1990s. We have designed a modular method to assemble a core/shell NP structure which was named LPD [1–4]. The negatively charged DNA–protamine core allows subsequent wrapping by cationic liposomes. The resulting positively charged NP were further modified by inserting double-chain phospholipid conjugate of polyethylene glycol (PEG) to impart a steric barrier for prolonged circulation time in the blood. The distal end of PEG is tethered with a small targeting ligand such as anisamide for rapid and specific internalization of the bound NP [5]. Although LPD showed a striking success in delivering siRNA after intravenous injection, the release of siRNA into the cytoplasm is variable depending on the cells and still has room for improvement. Large molecular weight carbohydrate, hyaluronic acid, has been used to replace the DNA in LPD formulation which caused immunotoxicity [6].

Calcium phosphate (CaP) is a well used non-viral vector for *in vitro* transfection of a wide variety of mammalian cells with little toxicity [7]. The delivery activity is probably related to the fact that CaP rapidly dissolves in the acidic pH [8] Endocytosed CaP is expected to de-assemble in the endosomes and release its cargo into the cytoplasm. Many investigators, including ourselves, have attempted to improve the manufacture of the CaP precipitate with limited success [9]. Many other approaches have been attempted to prepare nano-sized CaP particles to improve the transfection reproducibility and efficiency. For example, Maitra and colleagues condensed DNA in a reverse micro-emulsion environment to prepare CaP NPs of 100–120 nm in diameter [8,10,11]. The colloids aggregate rapidly with time and only *in vitro* transfection has been reported. Epple et al. developed a formulation of CaP NPs by rapid precipitation followed by immediate adsorption of DNA or siRNA, which also stabilized the CaP colloids [12–14]. The stabilized NPs showed 100–200 nm in size and could transfect cells with a GFP plasmid or silenced the expression of GFP in the treated cells. Liu et al. reported a preparation of 24–35 nm CaP NPs coated with bovine serum albumin as a DNA vector *in vitro* [15]. However, the formulation was not tested via systemic administration. Thus, it would be a great improvement if one could combine the advantage of LPD and CaP NP to achieve a prolonged circulation time and an elevated endosome release mechanism.

Thus, we have replaced the core of LPD NP with the acid-sensitive CaP. The resulting new formulation is called Liposome/Calcium/Phosphate, or LCP. The CaP core should dissolve in the endosomes, causing osmotic swelling to release the encapsulated siRNA. After further modification of a

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