Tri-layer Micelles for Combination Delivery of Drug/siRNA

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ABSTRACT SUMMARY

A tri-layer polymeric micelle system based on the self-association of the cationic triblock copolymer poly(ethylene glycol)-b-poly[N-[N-(2-aminooethyl)-2-aminoethyl] aspartamide]-b-poly(e-caprolactone) (PEG-b-PAsp(DET)-b-PCL) has been synthesized and investigated for combination delivery of rapamycin and siYB-1. This system may potentially help improve the simultaneous delivery of combination siRNA/drug therapies.

INTRODUCTION

Polymeric micelle-based drug delivery systems (DDS) are attractive therapeutic modalities for treating cancer and genetic diseases because they allow for tumor targeting and controlled release of a single drug. For example, rapamycin (RAP) is a potent anti-cancer drug against many solid tumor types including breast, prostate, colon, and renal cell carcinomas. The mechanism of RAP involves inhibiting mammalian target of rapamycin (mTOR), which is a central regulator of cell growth and proliferation, leading to the cell growth arrest in G1 phase. Despite its potency in treating cancers, its clinical application is limited due to low aqueous solubility. Moreover, single drug therapy is often hindered by drug resistance that develops in cancer cells.

Delivery of small interfering RNA (siRNA) to cells is one effective approach to silence these resistance genes, often successfully re-sensitizing cancer cells to anticancer drugs. Y-box binding protein-1 (YB-1) targeted siRNA (siYB-1) has attracted great interest as a therapeutic agent because of its ability to efficiently knock down genes associated with cell proliferation and multiple drug resistance in various cancer cell lines. The simultaneous delivery of RAP and siYB-1 for combination anticancer therapy is very challenging. Therefore, to address this issue, we are investigating a novel polymeric micelle system based on the self-association of the triblock copolymer PEG-b-PAsp(DET)-b-PCL, as a nanocarrier of siYB-1/RAP. A major advantage of this system is biocompatibility since poly(ethylene glycol) (PEG) and poly(e-caprolactone) (PCL) are both approved by the FDA. Furthermore, PCL has been shown to encapsulate RAP with high loading efficiencies. The poly(β-benzyl-L-aspartate) (PBLA) portion of the backbone can be easily conjugated with many types of amine groups such as 1,2-diethylenetriamine (DET). DET contains amino groups that can protonate over a wide pH range and this is believed to help increase endosomal escape of nanoparticles via the proton sponge effect. In the structure of polymer, the hydrophilic and nonionic PEG block forms the outer layer to enhance the biocompatibility of the nanoparticles in vivo; the intermediate layer consists of the cationic DET segment which will be used to condense siRNA; the inner layer is made up of the hydrophobic and biodegradable PCL block which will be used to encapsulate RAP. This system may be potentially employed to improve the simultaneous delivery of combination siRNA/drug therapies.

EXPERIMENTAL METHODS

Diblock copolymer PEG-b-PBLA was synthesized by the ring-opening polymerization of β-benzyl-L-aspartate N-carboxy-anhydride (BLA-NCA) initiated by PEG-NH₃ (MW 5KDa) as previous reported, and this diblock copolymer was used as an initiator for the ring-opening polymerization of e-caprolactone to generate PCL. The precursor triblock copolymer PEG-b-PBLA-b-PCL was conjugated via aminolysis to DET through the PBLA side chain, generating the final cationic triblock copolymer. All the polymers at every step were characterized by ¹H-NMR and gel permeation chromatography (GPC).

Micelles were prepared by dissolving the triblock copolymer in 1ml acetone and quickly adding 1ml ddH₂O was while vigorously stirring the polymer/acetone solution. Acetone was then removed by evaporation in the hood, followed by centrifugation at 10, 000 rpm for 5 min, and then passed through a 0.2 μm Nylon syringe filter. RAP-loaded micelles and siRNA/RAP-loaded micelles were prepared in the same way with the addition of RAP and/or RAP with or without siRNA.

Particle size was characterized by dynamic light scattering (DLS) and the Zeta potential of micelles were measured with a Zetasizer Nano-ZS (Malvern Instruments, UK). Gel retardation and EtBr exclusion assays were performed to confirm the ability of cationic triblock copolymer to condense total RNA (tRNA). RAP release profiles from RAP-loaded micelles and tRNA/RAP-loaded micelles were conducted by dialyzing against ddH₂O for 24 hrs.

The gene silencing efficiency of polyion complexes (PICs) formed from the triblock copolymer with siRNA against luciferase (siLuc) was evaluated in LNCaP-Luc prostate cancer cells.

In vitro cytotoxicity of the combination siYB-1/RAP-loaded micelles was investigated in PC3 cells and cell viability was determined by a resazurin dye assay, as previously reported.

RESULTS AND DISCUSSION

The cationic triblock copolymer PEG-b-PAsp(DET)-b-PCL was synthesized via a two-step ring-opening
polymerization (as illustrated in Scheme 1) and number of repeating units was determined by integrating ratio of proton peaks through 

$^{1}H$-NMR, which was found to be 36 for pAsp(DET) and 88 for PCL; the triblock copolymer therefore having a total molecular weight of 22 KDa.

Scheme 1. Synthesis of PEG-b-pAsp(DET)-b-PCL

DLS revealed that empty micelles averaged 70 nm in diameter, 81 nm when loaded with RAP (RAP/NP), 113 nm when loaded with siRNA (siRNA/NP), and 108 nm when loaded with both siRNA and RAP (siRNA/RAP/NP). Agarose gel electrophoresis demonstrated that the band of free tRNA completely retarded at N/P ratio of 2, indicative of electrostatic interactions between from the triblock copolymer and tRNA. The condensation degree of tRNA was quantitatively determined by EtBr exclusion assay. At N/P ratio of 2, the condensation degree was 85%, which is similar to that found for the control diblock copolymer PEG- b-PAsp(DET).

Figure 1. Viability of PC3 cells after 24 hr (A) or 48 hr (B) incubation with either polymer, siYB-1/NP, RAP/NP or siYB-1/RAP dual-loaded NP.

The gene silencing efficiency of siLuc/triblock copolymer PICs was 43%-59% at the N/P ratio of 10-50, whereas the gene silencing activity of PICs formed from siLuc/PEG-b-PAsp(DET) were not as effective. The results indicate that the triblock copolymer significantly improves the transfection efficiency of siLuc. This is most likely due to presence of the hydrophobic PCL core which helps stabilize PICs further.

To investigate the in vitro cytotoxicity of combination RAP and siYB-1 in PC3 prostate cancer cells, we looked at sequential and simultaneous dosings of the drugs at both 24 and 48 h time points (Fig. 1). It is important to note that at the polymer concentration investigated (6 µg/ml) there was no cytotoxicity to cells observed up to 48 h, with cell viability similar to untreated cells. When siYB-1/NP at 50 nM was administered to PC3 cells, we found that over >91% of cells were still viable after 48 h thus downregulation of YB-1 alone did not appear to be detrimental to PC3 cells. Because downregulation of genes in vitro by siRNA sometimes takes 24-72 h for full effect, it was necessary to downregulate YB-1 in cells with siYB-1/NP at least 24 h prior to treatment with the RAP formulations. The next day, RAP in DMSO (RAP/DMSO), RAP/NP, or dual loaded siYB-1/RAP/NP at 0.01, 0.1 or 1 nM final RAP concentrations were added to cells. After waiting another 24 and 48 h, the cell viability was measured. We found that cell viability decreased significantly at 48 h for dual loaded siYB-1/RAP/NP. For example, we obtained similar results for RAP/DMSO and RAP/NP formulations at 24 and 48 h, whereas dual-loaded NP cell viability decreased significantly from 24 to 48 h at all RAP concentrations tested: from 81% to 42% for 0.01 nM RAP (p<0.001), 65% to 45% for 0.1 nM RAP (p<0.001), and 67% to 38% for 1 nM RAP (p<0.001), respectively.

CONCLUSION

A novel polymeric micelle system based on the cationic triblock copolymer PEG-b-PAsp(DET)-b-PCL has been developed for the simultaneous delivery of siYB-1 and RAP. Presence of PCL in the triblock copolymer has been found to improve the transfection efficiency of siLuc compared to the diblock copolymer PEG-b-PAsp(DET). This system has shown promising results in investigations into combination therapies, notably RAP and siYB-1.

REFERENCES


ACKNOWLEDGMENTS

We would like to acknowledge the University of Wisconsin-Madison School of Pharmacy for startup funds to support this work and for TA support (S. Zeng).