Intracellular Delivery of Biologic Drugs

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ABSTRACT SUMMARY
Multi-functional carriers for nucleic acids and proteins have been developed for nucleic acid and protein drugs that enhance endosomal release and cytosolic delivery to disease targets. The carrier systems are designed to mechanistically mimic pathogenic delivery systems that have evolved remarkably efficient cytosolic delivery capabilities. After internalization into vesicular compartments, the pH drops during endosomal-lysosomal development, triggering exposure of a membrane-destabilizing domain in viral proteins such as hemagglutinin or pathogenic proteins such as diphtheria toxin. The ampholytic carriers are designed like these pathogens to activate via protonation events triggered in the endosome. This endosomal-releasing activity is then built into a multi-functional polymer platform that incorporate targeting elements, conjugation or complexation elements, and a “stealth” component to optimize safety and pharmaco-kinetic properties.¹⁻⁵

INTRODUCTION
Nucleic acid and protein biologic drugs are currently limited in scope by delivery barriers at the system, tissue, and sub-cellular levels. Synthetic polymeric delivery systems have been developed that mimic the highly efficient intracellular delivery systems found in pathogenic viruses and organisms. The carriers possess a hidden functionality that is expressed in the endosomal compartment to increase cytosolic delivery of macromolecules. Controlled polymerization techniques are exploited to streamline bioconjugation of targeting agents and therapeutics, as well as to generate controlled carrier architectures.

EXPERIMENTAL METHODS
Polymeric carrier synthesis and characterization: RAFT controlled radical polymerization was used to synthesize diblock copolymers with an outer stealth, corona-forming component, a pyridyldisulfide monomer that allows conjugation of thiolated biologic drugs, and contains a conjugation linkage for targeting agents. Polymers were analyzed by ¹H NMR to assess purity and gel-permeation chromatography was used to determine number average (Mn) molecular weight and polydispersity (PDI).
Nucleic acid and protein conjugation via thiol-exchange: The degree of biologic drug conjugation to the block copolymer was determined by measuring the release of pyridine-2-thione at 343 nm using an extinction coefficient of \(8.08 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}\). To determine the polymer to thiol ratio at which complete drug conjugation occurs, gel retardation assays are conducted at varying molar ratios of PDS groups to thiolated drug.
Cellular uptake and intracellular distribution of the antibody-targeted pro-apoptotic peptide-polymer conjugates: To help determine the optimal the peptide to polymer stoichiometric ratio in relation to cell targeting activities, fluorescence microscopy and radiolabel/cell fractionation experiments was conducted in living cells as previously described.⁵ FACS analysis was used to quantitate targeting and uptake efficiencies using fluorescently labeled peptides/proteins.
Carrier safety: Safety analysis was conducted after polymer micelle administration via tail vein injection. Blood was collected on day 1, 3, and 5 to measure toxicity through blood enzyme and organ histology characterization.
PK characterization: For in vivo studies, mice
were housed and treated under protocols approved by the FHCRC and UW Institutional Animal Care and Use Committees. For PK, serial blood samples were drawn in triplicate from the retro-orbital venus plexus with timepoints at 5 m, 15 m, 30 m, 1 h, 2 h, 4 h, 8 h, and 24 h. Concentrations were plotted using GraphPad Prism and plotted to a two-compartmental model.

**Therapeutic activity:** Mice bearing tumor xenografts were treated with active drug carriers and controls. The weights of mice and sizes of the tumors were measured every 2 days. For vaccine studies, mice were injected subcutaneously at the base of the tail on days 0 and 21. One week post boost immunization (day 28), mice were sacrificed and spleens harvested to assess the cellular immune response as described by Wilson et al.²

**RESULTS AND DISCUSSION**

The pH-responsive diblock copolymers enhance protein delivery to pro-apoptotic targets in cancer cells. The polymers strongly enhance protein delivery to the cytoplasmic compartment in living cancer cells. The polymers also enhance PK properties (blood half-life) and have excellent safety profiles as assessed by organ histology and organ enzyme analysis. Antibody-targeted protein drugs significantly reduce tumor growth rates and enhance survival times by Kaplan-Meier analysis. This therapy effect was shown to correlate with target-induced induction of cancer cell apoptosis.

For vaccine delivery, conjugation of antigen to polymer micelles significantly enhanced antigen cross-presentation in vitro. Mechanistic studies demonstrated this enhancement was correlated with micelle-mediated enhancements in intracellular antigen retention and cytosolic antigen accumulation. Subcutaneous immunization of mice with ovalbumin-polymer conjugates significantly enhanced antigen-specific CD8⁺ T cell responses (0.4 % IFN-γ⁺ of CD8⁺) compared to immunization with soluble protein, antigen and polymer mixture, and the control micelle without endosome-releasing activity. Additionally, pH-responsive carrier facilitated antigen delivery to antigen presenting cells in the draining lymph nodes. These results demonstrate the potential of pH-responsive polymeric micelles for use in vaccine applications that rely on CD8⁺ T cell activation.

**CONCLUSION**

The carriers are applicable to a wide range of biotherapeutics, and might open up new families of peptide, antibody or nucleic acid drug candidates that attack previously inaccessible intracellular targets. Applications include intracellular protein drugs, siRNA drugs, and protein-based vaccines for cancer therapies.

**REFERENCES**


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