Biomimetic Drug Delivery Systems for Biologic Drugs

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Statement of Purpose: The long-term objective of our research is to develop new drug delivery systems for biologic drugs that must reach intracellular targets for efficacy. Barriers include drug stability, tissue penetration and transport, and therapeutic targeting, but cytoplasmic entry is a widespread barrier for those that function intracellularly. Synthetic polymeric carriers have been developed that mimic the highly efficient intracellular delivery systems found in pathogenic viruses and organisms. Their most important property ties together the sensing of pH changes to membrane destabilizing activity, and the carriers thus possess a hidden functionality that is expressed in the endosomal compartment to increase cytosolic delivery of macromolecules. Another important aspect of these polymeric carriers is the development of controlled polymerization techniques (1) to streamline bioconjugation of targeting agents and therapeutics, as well as to generate controlled carrier architectures. 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.

Methods: For peptide delivery, a pyridyl disulfide functionalized CTA[3] was synthesized and used to make a pyridyl disulfide □-functionalized diblock copolymer. The first block was polymerized with N-(2-hydroxypropyl) methacrylamide (HPMA), and the resultant HPMA macro-CTA was used for subsequent block copolymerization of dimethylaminoethyl methacrylate (DMAEMA), propylacrylic acid (PAA), and butyl methacrylate (BMA). Peptide conjugation was performed with a cell-internalizing Bak-BH3 peptide (ant-Bak-BH3) (2)containing a carboxy-terminal cysteine. This thiol-containing amino acid was reacted with the pyridyl disulfide functionalized polymer and SDS-PAGE gels were utilized to confirm peptide-polymer conjugation. Cell death was determined using a lactate dehydrogenase (LDH) cytotoxicity detection kit. For RNA delivery, polymers were synthesized using RAFT polymerization, resulting in diblocks where the first block (DMAEMA) molecular weight was 9100 kDa and the second block (PAA-co-DMAEMA-co-BMA) was 20.5 kDa composed of 23% PAA, 24% DMAEMA, and 53% BMA.

Results: The pyridyl disulfide CTA was synthesized as verified by NMR analysis. The diblock polymer (**M**n 19,000 g/mol, PDI 1.27) was composed of an**N**-(2-hydroxypropyl) methacrylamide (HPMA) first block (**M**n 13,800 g/mol, PDI 1.13) intended to enhance water solubility and circulation time. The second polymer block was a pH-responsive composition designed to enhance endosomal escape and consisted of equimolar quantities of dimethylaminoethyl methacrylate (DMAEMA), propylacrylic acid (PAA), and butyl methacrylate (BMA). Thiol-disulfide exchange reactions were found to efficiently produce reversible polymer conjugates (75 mol % peptide reactivity with polymer) with the cell-internalized proapoptotic peptide. Peptide-polymer conjugates also produced significantly increased apoptotic activity over peptide alone in HeLa cervical carcinoma cells as found using flow cytometric measurements of mitochondrial membrane depolarization (2.5-fold increase) and cell viability tests that showed 50% cytotoxicity after 6 h of treatment with 10 **μ**Mpeptide conjugate. These results indicate that this multifunctional carrier shows significant promise for proapoptotic peptide cancer therapeutics and also as a general platform for delivery of peptide drugs with intracellular targets. (3)

The RNA carriers were composed of a positively charged block of dimethylaminoethyl methacrylate (DMAEMA) to mediate siRNA binding and a second pH-responsive endosome releasing block composed of DMAEMA and propylacrylic acid (PAA) in roughly equimolar ratios and butyl methacylate (BMA)[4]. These polymers spontaneously form spherical micelles in the size range of 40 nm with CMC (critical micelle concentration) values of approximately 2 **μ**g/mL based on dynamic light scattering (DLS), 1H NMR, electron microscopy, and selective partitioning of the small molecule pyrene into the hydrophobic micelle core. Under these conditions, the micelle-based systems showed an 89% reduction in GAPDH mRNA levels as compared to only 23% (10 nM siRNA) for the nonmicelle system. The reduction in mRNA levels becomes nearly quantitative as the siRNA concentration is increased to 25 nM and higher. Flow cytometry analysis of fluorescent-labeled siRNA showed uptake in 90% of cells and a 3-fold increase in siRNA per cell compared to a standard lipid transfection agent.

Conclusions: pH-responsive carriers can enhance cytosolic entry and activity of RNA and other biologic drugs, validating their further preclinical development for a variety of disease therapeutics.

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References

1. J. Chiefari, Y. Chong, et al. Macromolecules 31, 5559–5562 (1998)

2. E.P. Holinger, T. Chittenden, and R.J. Lutz. J. Biol. Chem. 274, 13298–13304 (1999).

3. C. Duvall, A. Convertine, D. Benoit, A. Hoffman, P. Stayton, Mol. Pharm., 7:468-76 (2010).

4. A. Convertine, C. Diab et al., Biomacromolecules 2010, 11, 2904–2911

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