157c An Anionic Polymer Enhances Cationic Lipid-Mediated Delivery of Antisense Oligonucleotides

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The use of antisense oligonucleotides (AS ODNs) to inhibit the expression of specific mRNA targets represents a powerful bioengineering strategy for inhibition of gene expression. Cationic lipids and polymers are frequently used to improve the delivery of AS ODNs to cells, but the resulting complexes often aggregate, bind to serum components, and are trafficked poorly within cells. Crucial to the success of antisense downregulation is the escape of the vector from the endosomal pathway, accompanied or followed by release of oligonucleotides so that they may hybridize to their mRNA targets [Roth and Sundaram]. In light of this, some synthetic vectors are formulated with endosomal escape moieties that become protonated upon endosomal acidification, hence promoting membrane disruption and subsequent release of DNA into the cytoplasm.

Previously, it was shown that a synthetic, pH-sensitive, membrane-disrupting polyanion, poly(propylacrylic acid) (PPAA) was able to enhance delivery of plasmid DNA by the commercial cationic lipid dioleoyl trimethylammonium propane (DOTAP) both in vitro and in vivo [Kyriakides et al.]. While improved delivery of plasmids has been demonstrated by combining PPAA with DOTAP, this system has not been studied with respect to oligonucleotide delivery. Although plasmid DNA and oligonucleotides each interact electrostatically with cationic liposomes to self-assemble into complexes, the considerable disparity in their molecular weights and topologies may influence the vector properties and therefore their cellular activity. The experience obtained from gene therapy could help to address issues regarding antisense oligonucleotide delivery. In light of this, we have investigated the extent to which PPAA can enhance delivery of antisense oligonucleotides.

We show that the addition of PPAA improves the in vitro efficiency of DOTAP with regards to oligonucleotide delivery and antisense activity. In characterization studies, ODN complexation in DOTAP/ODN complexes was preserved even when substantial amounts of PPAA were added. The formulation also exhibited partial protection of phosphodiester oligonucleotides against enzymatic digestion. In both CHO and A172 cells, incorporation of PPAA in DOTAP/ODN complexes improved two- to threefold the cellular uptake of fluorescently labelled oligonucleotides. DOTAP/ODN complexes containing PPAA also maintained high levels of uptake into cells upon exposure to serum. Addition of PPAA to DOTAP/oligonucleotide complexes also enhanced the antisense activity (using GFP as the target) over a range of PPAA concentrations in both serum-containing and serum-free media. Hence, this novel and simple system could, with further study, aid the use of cationic lipids for DNA delivery.

References

Kyriakides TR, Cheung CY, Murthy N, Bornstein P, Stayton PS, Hoffman AS (2002) pH-sensitive polymers that enhance intracellular drug delivery in vivo. J Control Release 78(1-3): 295-303.

Roth CM, Sundaram S (2004) Engineering synthetic vectors for improved DNA delivery: insights from intracellular pathways. Annu Rev Biomed Eng 6: 397-426