CLINICAL TRIALS AND TRANSLATIONAL MEDICINE COMMENTARY

Drug Delivery Trends in Clinical Trials and Translational Medicine: Challenges and Opportunities in the Delivery of Nucleic Acid-Based Therapeutics

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ABSTRACT: The ability to deliver nucleic acids (e.g., plasmid DNA, antisense oligonucleotides, siRNA) offers the potential to develop potent vaccines and novel therapeutics. However, nucleic acid-based therapeutics are still in their early stages as a new category of biologics. The efficacy of nucleic acids requires that these molecules be delivered to the interior of the target cell, which greatly complicates delivery strategies and compromises efficiency. Due to the safety concerns of viral vectors, synthetic vectors such as liposomes and polymers are preferred for the delivery of nucleic acid-based therapeutics. Yet, delivery efficiencies of synthetic vectors in the clinic are still too low to obtain therapeutic levels of gene expression. In this review, we focus on some key issues in the field of nucleic acid delivery such as PEGylation, encapsulation and targeted delivery and provide some perspectives for consideration in the development of improved synthetic vectors. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:38–52, 2011

Keywords: biotechnology; cationic lipids; DNA/oligonucleotide delivery; encapsulation; gene delivery; gene vectors; lipoplexes; macromolecular drug delivery; nanoparticles; nonviral gene delivery

INTRODUCTION

The development of nucleic acid-based therapeutics has garnered tremendous interest in the past two decades as a new category of biologics. The ability to deliver nucleic acids (e.g., plasmid DNA, antisense oligonucleotides, siRNA) offers the potential to develop potent vaccines and novel therapeutics to cure many diseases that are difficult to treat effectively with traditional therapies, for example, hereditary diseases, cancer.[1–4] However, the site of action of nucleic acids requires that these molecules be delivered to the interior of the target cell, which greatly complicates delivery strategies and compromises efficiency. The poor transfection efficiencies of synthetic delivery systems (nonviral vectors, i.e., complexes of polynucleotides with cationic lipids and/or polymers) has forced the majority of clinical trials to employ viral vectors despite the significant safety concerns associated with their immunogenicity and insertional mutagenesis.[5,6] Although nonviral vectors proficiently transfect cells in culture, it must be appreciated that most cell culture experiments employ rapidly dividing cells cultured in monolayers that do not accurately mimic the *in vivo* situation. Under these conditions, precipitation can enhance the association of the delivery system with the cell surface, which can artifactually elevate transfection rates with delivery systems that are not stable in physiological media. Conversely, delivery systems that are stable in physiological media often do not transfect efficiently in cell culture, leading to the mistaken conclusion that such systems are not worthy of further consideration. Another confounding factor with cell culture experiments is that the nuclear membrane breaks down during cell division, allowing efficient translocation of DNA into the nucleus of rapidly dividing cells that greatly facilitates transfection. These differences between cell culture and *in vivo* gene delivery misled several companies who employed combinatorial chemistry to

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create tens of thousands of novel delivery agents in the 1990s, only to screen for efficacy in cell culture. Unfortunately, these massive efforts yielded minimal insight into the structure–function relationship of delivery agents that is relevant to the *in vivo* situation. Similar approaches have recently been employed to develop delivery agents for siRNA, and some of the identified "lipidoids" have shown promise in nonhuman primates.[7,8] Currently, delivery efficiencies of synthetic vectors in the clinic are too low to obtain therapeutic levels of gene expression. As of December 2009, there have been 1579 gene therapy clinical trials worldwide, of which about 25% have utilized nonviral vectors.[9] It should be noted that viral vectors still dominate clinical gene therapy, with the first approved gene therapy product, Gendicine, being the Recombinant Human Ad-p53.[10] Besides the approved product in China, some of the viral vector-based therapeutics have progressed to phase III clinical trials,[11–13] such as Rexin-G, a tumor-targeted retrovirus bearing a cytocidal cyclin G1 construct and ONYX-015, an oncolytic adenovirus. While viruses offer greater efficiency of gene delivery, it is generally agreed that synthetic vectors would be preferable due to safety concerns, and viral vectors may be more suited for *ex vivo* applications.[14] It is clear that the strategies employed by these two classes of vectors appear to be converging,[15] but the challenges associated with intracellular delivery are significant. In this review, some key issues in the field of nucleic acid delivery will be addressed for consideration in the development of improved synthetic vectors. Considering that cationic liposomes are the best studied of the nonviral delivery systems, much of the discussion will review findings with these systems, but these issues are also relevant to polymeric systems.

DNA VERSUS siRNA

Cationic liposomes and polymers are widely used as nonviral vectors both *in vitro* and *in vivo*. Both plasmid DNA and siRNA bind to cationic liposomes and polymers via electrostatic interaction between the anionic phosphodiester backbones and the positively charged group in cationic delivery agents. Indeed, it was recognized by Papahadjopoulos and coworkers[16–18] that encapsulation efficiency of nucleic acids within traditional anionic liposomes was low, and thus cationic lipids were synthesized in an attempt to improve loading efficiency and promote cellular uptake. The application of this idea by Felgner et al.[19] in 1987 stimulated immense interest in developing nonviral vectors for therapeutic use. At the time of this landmark paper, the mechanism of RNA interference had yet to be discovered, but cationic agents that bind DNA would be expected to interact with RNA via similar electrostatic interactions with the phosphate backbone.[20,21] However, it is important to recognize the significant structural differences between plasmid DNA and siRNA. The most obvious difference is that plasmid DNA is typically 5000 base pairs or larger whereas siRNA is typically 20–25 bp; a 200-fold difference in molecular weight. Work from our laboratory has shown that the affinity of cations for short deoxy-oligonucleotides can vary substantially by sequence, and is significantly different from that to plasmid DNA.[22] Furthermore, the interaction of plasmids with some multivalent cationic agents causes a remarkable reduction in molecular volume classically referred to as "condensation."[23–25] This event is characterized by a molecular collapse that protects the DNA from nucleases and chemical degradation, but true condensation is limited to polynucleotides greater than approximately 400 bases.[26] DNA condensation has been exploited by Copernicus Therapeutics to collapse single molecules of plasmid DNA into very small structures that facilitate translocation across cellular barriers.[27,28] Unfortunately, the term "condensation" is frequently misused in the delivery literature to describe the electrostatic association of nucleic acids with a delivery vehicle regardless of polynucleotide size, valency of the cation, or ability of the delivery system to affect molecular collapse. Although the complexation of poly- and oligo-nucleotides with cationic delivery vehicles typically results in nuclease resistance, condensation is rarely achieved.

In addition to molecular size, it is important to realize that DNA assumes a B conformation in physiological solutions in contrast to RNA, which assumes a less-hydrated, more compact A conformation characterized by a relatively narrow major groove and shallow minor groove. It follows that these structural differences that alter the spacing of phosphates in the backbone might also affect the interaction of cations with siRNA. This idea is consistent with experiments showing a dramatically reduced binding stoichiometry of cations for ribo- versus deoxyribo- oligonucleotides (20 bp), which may be relevant to incorporation into cationic delivery systems.[29] Furthermore, a reduced binding of cationic agents to RNA could potentially explain why much higher levels of cationic agents (i.e., +/− ratio) are generally needed to deliver RNA as compared to DNA.[30] Reduced interactions of RNA with cationic agents could also play a critical role in the release of siRNA from the delivery vehicle into the cytoplasm, which would influence the ultimate biological effect.

Aside from the physicochemical differences between DNA and RNA, it should be noted that plasmid DNA needs to be transported into the nucleus for gene expression, which requires crossing two