

Editorial

Plasmid-Based Gene Therapy: Opportunities and Challenges Knock at the Millennium

RAM I. MAHATO*

Department of Pharmaceutics and Pharmaceutical Chemistry, Center for Controlled Chemical Delivery (CCCD), University of Utah, Biomedical Polymers Research Building, 20S 2030E, Rm. 205, Salt Lake City, UT 84112-9452, USA

(Received 20 September 1999; In final form 21 September 1999)

"We cannot climb Mount Everest overnight! It is only by examining and reexamining our findings and ideas that we can turn genes into gene medicines."

As we approach the new millennium, one looks back and asks, "What went wrong? Where are we today and where would we like to be in the future?" In this series of Theme Issues in the *Journal of Drug Targeting*, we attempt to illustrate the recent advances and bottlenecks in plasmid-based gene therapy and what we can learn from viral vectors. The articles in this issue can be broadly categorized into the following distinct arenas: (1) intellectual property and commercialization of gene medicines; (2) cationic lipid, polymer and peptide-based systems; (3) microspherical systems; and (4) adeno-associated viral (AAV) vectors. This is by no means a comprehensive collection but provides a basis for further reading.

Gene therapy is a method for the prevention, correction or modulation of genetic and acquired diseases that uses genes to produce therapeutic proteins. Gene therapy present unique challenges and opportunities that compel us to consider a new clinical paradigm. Despite the renewed optimism of gene therapy, proof-of-concept in humans and eventual commercialization of gene medicines has not yet been accomplished (Wilson, 1999). Ben McGraw overviews the challenges facing the development of gene medicines and the need to change the poor perception about gene therapy by the media, public and financial community. Intellectual property protection of products and processes through patents is essential in the field of gene therapy. Almost all dedicated gene therapy companies do not have any products in the market and thus much of their values lies in the potential worth of their patent portfolio (Martin and Thomas,

* Tel.: +(801) 581 6654. Fax: +(801) 581 7848. E-mail: rmahato@hotmail.com.

1998). Mike Fons discusses that unlike conventional and protein drugs, gene medicines consist of multiple elements, each of which is likely to be covered by issued patents.

The development of effective gene medicines requires multi-disciplinary approach, which requires team work among scientists with different expertise, such as molecular and cell biology, biochemistry, biophysics, polymer science, colloid science, pharmaceuticals and medicine. In the design of gene delivery systems, scientists have used synthetic reagents that emulate components of viruses as models for efficient plasmid delivery. As described by us, synthetic peptide-based gene delivery systems that incorporate four viral attributes, such as DNA condensing, receptor-binding, endosome-disrupting, and nuclear-targeting elements are the least developed, but can be designed to be well-defined structural and chemical properties and functions (Mahato *et al.*, 1999).

Benn and Kim discuss the 'terplex' system, which consists of stearyl-poly(L-lysine) (PLL), low-density lipoprotein (LDL) and plasmid DNA. The 'terplex' system uses a balance of charge and hydrophobic interactions to stabilize DNA particles for their *in vivo* applications. Kabanov and associates discuss the possible use of block and graft copolymers and nanogel copolymer for gene delivery. Hennink and colleagues studied the effect of tertiary amino groups versus quaternary amino groups on gene transfer by comparing poly(2-dimethyl-amino)ethyl methacrylate (pDMAEMA) and its quaternary ammonium analogue poly(2-trimethyl-amino)ethyl methacrylate (pTMAEMA). These authors demonstrated that quaternization of polycation decreased transfection efficiency compared to that obtained using non-quaternarized polyamine analogue. This suggests that protonation of polyamines in the endosomes is essential for destabilization of the endosome, release of DNA or DNA/polycation complexes in the cytoplasm and translocation of DNA into the nucleus for gene expression. Wagner and associates demonstrated that transferrin-polyethyleneimine (PEI) conjugates can efficiently transfer transgenes to

melanoma cells both *in vitro* and *in vivo*. Lollo and associates demonstrate that PEGylation of PLL and addition of Brij 35, a surfactant, to PLL/DNA complexes significantly improve transgene expression in the liver after systemic administration. Urtti and associates evaluated polycations and cationic lipids for gene delivery to human retinal pigment epithelial cells *in vitro*. Hashida and associates assessed the *in vivo* disposition characteristics of mannosylated-PLL/plasmid complexes after intravenous injection into mice. Hedley and associates studied the effect of process parameters on encapsulation of plasmid DNA into poly(D,L-lactide-co-glycolide) (PLGA) microspheres for gene delivery to antigen presenting cells.

Liu and associates synthesized a series of novel aromatic ring-based cationic lipids and characterized them for gene delivery. Cullis and associates reported on 'stabilized plasmid-lipid particles' containing the fusogenic lipid dioleoylphosphatidylethanolamine (DOPE), cationic lipid dioleoyldimethylammonium chloride (DODAC), and stabilized by a polyethyleneglycol (PEG) coating. The PEG moieties are attached to a ceramide anchor. These DNA particles are fairly stable in serum, have prolonged plasma half-life and produce enhanced gene expression in tumor. Cheng and associates discuss the influence of cationic lipid structures and formulation parameters for optimal gene transfer to the lung. The structure and protonation of cationic head-groups as well as the type and ratios of neutral co-lipid are important parameters that determine the overall activity of formulated plasmid in the lung. There was a significant increase in gene expression when the free base of cationic lipid containing T-shape (instead of linear) head-group was used. Systemic administration of lipid/plasmid complexes usually produces much lower levels of gene expression in subcutaneous tumor than in the lung metastases. In an attempt to enhance gene delivery and expression to distal tumors after systemic administration into subcutaneous tumor bearing mice, Anwer and associates optimized various formulation parameters, such as lipid/co-lipid ratio, liposome size, charge ratio and PEGylation.

Compared to viral vectors, transfection efficiencies of non-viral gene delivery systems are still very low, possibly due to poor understanding of the self-assembled structures of cationic carriers/plasmid complexes and their interactions with plasma membranes and the events leading to DNA release in the cytoplasm and translocation to the nucleus. Safinya and associates characterized the internal structure of lipid/plasmid complexes: a layered-structure formed from cationic lipid bilayers alternating with layers of parallel DNA strands. They demonstrated that strikingly different internal structures can form by lipid/plasmid complexes simply by substitution of co-lipid DOPC with DOPE. They also attempted to characterize these internal differences and correlate structural differences with gene transfer activity.

Transient gene expression of non-viral gene delivery systems remains to be one of the major obstacles for their clinical applications. Therefore, attempts are being made to prolong the duration of transgene expression of plasmid-based systems by incorporating some viral elements either in the delivery systems or in the plasmid construct itself. Demeneix and associates attempted to enhance the level and duration of gene expression by co-transfecting the reporter plasmids with an anti-apoptotic gene, such as CMV-bcl-X_L expression vector. These authors demonstrated that the loss of gene expression in mouse brain is due to cell death and gene expression can be prolonged by over-expression of an anti-apoptotic gene, which could block the activation of caspases during post-transfection.

A viral vector consists of a therapeutic gene that can be taken up by the target cell, leading to gene expression. To develop virus-like synthetic vectors, we need to have clear understanding of the key properties and limitations of viral vectors, such as retrovirus, adenovirus and AAV-based systems. Reed and associates discussed that AAV-based vectors have a way of inserting their genes into target cells without activating T-cells. Vector transduction was not associated with any cell-mediated immune response and there was a prolonged gene expression in terminally differentiated cells of central nervous systems.

I am grateful to all the authors for their contribution to this Theme Issue of *Journal of Drug Targeting*. I hope that this issue provides stimulation for innovative approaches to gene delivery and look forward to further development in this fascinating field. Despite early setback, there is no doubt that gene medicines will eventually be used as pharmaceuticals. We must maintain a strong competitive edge to allow individual accomplishment while working together in areas of mutual benefit.

References

- Mahato, R.I., Smith, L.C. and Rolland, A. (1999) Pharmaceutical perspectives of gene therapy. *Adv. Genet.* 41: 95-156.
- Martin, P.A. and Thomas, S.M. (1998) Special feature: The commercial development of gene therapy in Europe and the USA. *Hum. Gene Ther.* 9: 87-114.
- Wilson, J.M. (1999) Presidential address of American Society of Gene Therapy. <http://www.asgt.org>.