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Cationic Polymers and its Uses in Non-viral Gene Delivery Systems: A Conceptual Research

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Abstract: The main objective in gene delivery system is the development of efficient, non-toxic gene carriers that can encapsulate and deliver foreign genetic materials into specific cell types To perform gene transfer two types of vectors are available, (i) viral vectors (ii) nonviral vectors. Among the vectors, nonviral vectors are proved less toxic and safe compare to viral vectors through clinical trial. No single vector is proved suitable for every gene transfection experiment. Cationic polymers such as polyethylenimine (PEI) and poly L-lysine (PLL) and their complexes are experimentally established as non-viral vector with higher transfection efficiency. Identifying the barriers for transfection and the possible solution, the stability improvement research is needed for better clinical performance of cationic polymers. In future it is also possible to find new conjugates as cationic polymers bearing many properties, suitable to bound with DNA and penetrate cell. Considering all these properties, these works reviews the most recent studies highlighting cationic polymers used in nonviral gene delivery systems.

Key words: Gene delivery, cationic, PLL, PEI, polymer

Introduction

General Aspects

Gene Delivery System represents a new and promising therapeutic modality. The underlying principle is based on the introduction of genetic material into cells to generate a curative biological effect (Miller, 1992; Mulligan, 1993). Gene therapy is not limited to hereditary diseases but can be used for a broad variety of different acquired diseases, such as infections, degenerative disorders and cancer. The most challenging issues for successful application of gene therapy to human diseases concern: (1) the choice of the relevant therapeutic gene, (2) the choice of promoter and regulatory sequences driving expression of the transgene and (3) the vector used for delivery of the transgene into cells (target cells). Promoter, regulatory elements and vector characteristics determine transduction efficacy (the number of target cells expressing the transgene and the intensity of gene expression per cell), specificity of the transduction, time of transgene expression, the host's immune response against the vector and eventually undesired side effects. At present, important efforts are being focused on the search for vectors with less toxicity and prolonged and controlled transgene expression, thereby widening the potential application of gene therapy to a high spectrum of medical fields. In recent years, gene therapy has emerged as a new and promising method to treat human diseases. To perform gene delivery two types of vectors are available,

- Viral vectors (retorviruses, adeneviruses, herpesviruses, lentiviruses and hybrid/retor-adeno viruses etc).
- Nonviral vectors (cations e.g., polymers and lipids, naked DNA, artificial chromosomes, plasmid, conjugates and complexes).

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Despite of the impressive progress in biomedical sciences during the last decades, the therapy of many liver diseases remains unsatisfactory. This applies not only to primary and metastatic liver tumours, hepatic cirrhosis and hereditary metabolic diseases but also to a high proportion of cases with chronic viral hepatitis that do not respond to current antiviral therapy. There is evidently an urgent need for efficient alternative therapeutic approaches and in recent years gene therapy has emerged as a new and promising method to treat human diseases (Schmitz *et al.*, 2005)

Gene Therapy Vectors

Gene therapy vehicles can be categorized into two groups: biological and non-biological systems. Each group has its own advantage and limitations. The limitations of biological delivery system are less encountered in non-viral gene carrier namely cationic polymers, cationic peptides and cationic lipids (liposomes). The main objective in gene therapy is the development of efficient, non-toxic gene carriers that can encapsulate and deliver foreign genetic materials into specific cell types. During the past two decades, enormous research in the area of gene delivery has been conducted worldwide, in particular for cancer gene therapy application. Viral vectors are biological systems derived from naturally evolved viruses capable of transferring their genetic materials into the host cells. Many viruses including retrovirus, adenovirus, herpes simplex virus (HSV), adeno-associated virus (AAV) and pox virus have been modified to eliminate their toxicity and maintain their high gene transfer capability. The limitations associated with viral vectors, however, in terms of their safety, particularly immunogenicity and in terms of their limited capacity of transgenic materials, have encouraged researchers to focus on non-viral vectors as an alternative to viral vectors. Non-viral vectors are generally cationic in nature. They include cationic polymers such as polyethylenimine (PEI) and poly L-lysine (PLL), cationic peptides and cationic liposomes. The newly described liposomal preparation LPD (liposomes/protamine/DNA), for example, has shown superiority over conventional liposomes/DNA complexes (lipoplexes)(Aneed, 2004). However, physical properties such as size and zeta potential play a critical role in their efficiency. In either delivery system, selected modifications that can produce safe efficient and targetable gene carriers are desirable. Although non-viral vectors are less efficient than viral ones, they have the advantages of safety, simplicity of preparation and high gene encapsulation capability. This article reviews the most recent studies highlighting the advantages and the limitations of nonviral cationic types of gene delivery systems used in gene therapy (Aneed, 2004).

Both viral and non-viral vectors are used to transfer genetic material to the inside of target cells. In general, non-viral vectors have lower transduction efficiency and allow for shorter duration of transgene expression than viral vectors. The ideal vector should have (i) low antigenic potential, high capacity, high transduction efficiency, (ii) allow controlled and targeted transgene expression, (iii) reasonable expense and (iv) safe for both patient and the environment. Gene delivery vehicles must be selected according to the specific therapeutic aim. But the perfect vector covering all therapeutic and safety requirements does not exist and much work is needed in this important research field (Prince, 1998).

Non-viral Vectors

Non-viral systems are cationic in nature. They interact with negatively charged DNA through electrostatic interactions. The total charge however maintains a positive net value. This will enable the carrier of efficiently interacting with the negatively charged cell membranes and internalizes into the cell, which occurs maiuly through the endocytosis pathway (Behr, 1994).

The cationic natures of the non-viral vectors help the vector to interact with the negatively charged DNA through electrostatic interactions. The reaction complex also bears positive net values which enable the carrier to react efficiently with negatively charged cell membranes.

Nonviral gene transfer vectors have been actively studied in the past years in order to obtain structural entities with minimum size and defined shape. The final size of a gene transfer vector, which is compacted into unimolecular complexes, is directly proportional to the mass of the nucleic acid to be compacted. To perform gene delivery ssDNA (SS-Single stranded)/poly-L-lysine complex was found significantly smaller than their double-stranded counterparts. Expression of compacted ssDNA was observed in hepatoma cell lines. Firstly, galactosylated ssDNA complexes were successfully delivered into cells and then expression of the asialoglycoprotein receptor via receptor-mediated endocytosis. The reduced size and biophysical behaviour of ssDNA vectors may provide an advantage for transfection of eukaryotic cells (Behr, 1994).

Cationic Polymers

Synthetic cationic polymers combined with DNA at physiological pH and form a particulate complex (polyplex), capable of gene transfer into the targeted cells. As they are synthetic compound many modifications e.g., molecular weight and ligand attachment can be easily achieved. The most widely studied polymers for gene therapy include poly L-lysine (PLL) and polyethylenimine (PEI) (Aneed, 2004). Several polycations possessing sustainable buffering capacity below physiological pH, such as lipopolyamines and polyamidoamine polymers are efficient transfection agents, without the addition of membrane disruption agents. Indeed, every third atom of PEI is a protonable amino nitrogen atom, which makes the polymeric network an effective proton sponge at virtually any pH. Cytotoxicity was low and seen ouly at concentrations well above those required for optimal transfection. The PEI cation/anion balance for *in vitro* transfection is only slightly on cationic side, which is advantageous for *in vivo* delivery. Together these properties make PEI a promising vector for gene therapy and an outstanding core for the design of more sophisticated devices (Boussif *et al*., 1995).

Poly-L-lysine (PLL)

Basic Nature and Transfection Mechanisms

PLL polymers are one of the first cationic polymers employed for gene transfer (Wu and Wu, 1987). They are linear polypeptides with the amino acid lysine as the repeat unit; thus, they possess a biodegradable nature. This property is very useful for *in vivo* applications. Poly-L-lysine polyplexes are, however, rapidly bound to plasma proteins and cleared from the circulation (Ward *et al*., 2001; Dash *et al*., 1999). In addition, successful transfection requires co-application of chloroquine, a lysosomotropic agent, which reduces the lysosomal degradation of lipoplexes (Pouton *et al*., 1998; Shewring *et al*., 1997). The exact mechanism in which chloroquine acts is not well understood. Chloroquine can be substituted with fusogenic peptides, which undergo pH-related conformational changes perturbing the lysosomal/endosomal membranes, thus the DNA is successfully delivered into the cell cytoplasm (Lee *et al*., 2002; Wagner *et al*., 1992).

Poly-l-lysine Modified Complexes with Other Compounds

a) Poly-l-lysine Condensed Dna and Galactosylated Bovine Serum Albumin (Galbsa)

Liver targeting of DNA delivery could be enhanced via asialoglycoprotein receptors using a complex of poly-L-lysine (PLL) condensed DNA and galactosylated bovine serum albumin (GalBSA) via charge interaction (GalBSA:PLL:DNA = 3:0:5:1, w/w/w) (Han *et al*., 1999).

b) Poly-l-lysine Modified Iron Oxide Nanoparticles (IONP-PLL)

Another nonviral gene carrier is developed, which is poly-L-lysine modified iron oxide nanoparticles (IONP-PLL) and is formed by modifying poly-L-lysine to the surface of iron oxide nanoparticles. IONP-PLL could bind and protect DNA. In contrast to Poly-L-lysine and cationic

liposomes, ION-PLL proved less cytotoxic in a broad range of concentrations. Poly-L-lysine modified iron oxide nanoparticles can deliver exogenous gene to cells *in vitro* and *in vivo*. After intravenous injection, ION-PLL transferred reporter gene EGFP-C2 to lung, brain, spleen and kidney. Also it transfers exogenous DNA across the blood-brain barrier to the glial cells and neuron of brain. It is found a potential vector for gene therapy against CNS diseases (Xiang *et al*., 2003).

c) The Terplex Complex

A new DNA delivery vector (the terplex system) based on a balanced hydrophobicity and net surface charge between stearyl-poly-L-lysine, low density lipoprotein (LDL) and genetic material (i.e., plasma DNA or antisense oligonucleotide) was developed. The pSV-beta-gal plasmid in terplex system showed a 2-5 fold increase in betagalactosidase expression on murine smooth muscle cells (A7R5) compared to lipofection. Delivery of unmodified cmyb antisense oliogoneucleotide to A7R5 cells was also facilitated significantly by the terplex system requiring as little as 5.4 nm of antisense oligoneucleotide to achieve a 50% antiproliferative effect (Kim *et al*., 1998). After characterization of physical properties it is found that:

- Plasmid DNA was condensed by addition of stearyl-PLL and LDL, resulting in the terplex system of about 100 nm in diameter as shown by atomic force microscopy.
- A strong hydrophobic interaction between stearyl-poly-L-lysine and LDL was registered by 1H-NMR spectrometry, showing a significant decrease in the epsilon-methylene signal of poly-L-lysine backbone when stearyl-poly-L-lysine was mixed with LDL; however, this phenomena was not observed with unmodified poly-L-lysine.
- Agarose gel electrophoresis revealed that electrophoretic mobility of the terplex system decreased with increasing amounts of stearyl-poly-L-lysine, indicating that the surface charge of the terplex system became more positive by addition of stearyl-poly-L-lysine.
- Zeta potential measurement showed that the terplex system exerted a slightly positive charge (+2 mV) at a 1:1:1 weight ratio of plasmid DNA: LDL: stearyl-poly-L-lysine.

d) Poly-L-lysine-g-pluronic

By conjugating poly-L-lysine (PLL) to pluronic, poly-L-lysine-g-pluronic is synthesized. Pluronic is partially functionalized with para-nitrophenyl carbonate groups. This pluronic grafted poly-L-lysine is tested as a new synthetic gene carrier. Compared with unmodified PLL, PLL-g-pluronic showed about two fold increases in transfection efficiency with similar cytotoxicity specifically at the 1:1 weight ratio of polymer: DNA. The *in vitro* transfection efficiency was measured in Hela cells by using the O-nitrophenyl-beta-D-galactopyranoside assay (Jeon *et al*., 2003).

Efficiency Influencing Factors and Actions for Improvement

In fact, PLL has poor transfection ability when applied alone or without modifications (Pouton *et al*., 1998; Brown *et al*., 2000). One popular modification that can increase both the transfection ability and the circulation half-life of these vectors is coating with PEG (Lee *et al*., 2002; Ward *et al*., 2002). Targeting ligands were also linked to the polymerase chain (even in early studies) resulting in enhanced transfection (Wu and Wu, 1987; Suh *et al*., 2001). A common efficient strategy implies the addition of both PEG and a targeting ligand to PLL polymer to optimize transfection (Faraasen *et al*., 2003; Nah *et al*., 2002). Another approach can create the desirable proton sponge effect similar to that of PEI polyplexes by introducing histidine residues to PLL backbone (Pichon *et al*., 2001). Histidylated PLL showed better transfection efficiency than PLL/chloroquine mixture (Midoux and Monsigny, 1999).

Many PLL polymers with different molecular weight were tested and evaluated for gene transfer (Ward *et al*., 2002; Mannisto *et al*., 2002; Nishikawa *et al*., 1998). It has been shown that DNA condensation and transfection efficiency increased with high molecular weight PLL, which was also associated with undesirable high toxicity (Wolfert *et al*., 1999). The creation of amphiphilic PLL, by linking both PEG and palmitoyl groups to the polymer, reduced toxicity without compromising the gene delivery efficiency (Brown *et al*., 2000).

Polyethylenimine (PEI)

Basic Nature and Transfection Mechanism

PEI was used in gene delivery more recently than PLL. PEI is obtained by acid-catalyzed polymerization of aziridine (Dick and Ham, 1970; Klotz *et al*., 1969) yielding a highly branched network with a high cationic charge-density potential that can ensnare DNA. Since 1995, PEI has been found to be a versatile polymeric vector for gene delivery that tightly condenses plasmid DNA and is able to promote transgene delivery to the nucleus of mammalian cells (Boussif *et al*., 1995; Pollard *et al*., 1998). A merely mechanistic study (Wiethoff *et al*., 2001) has shown that cationic lipid-DNA complexes can dock and interact with proteoglycans expressed on the surface of mammalian cells, promoting their intracellular uptake, a possible route that might also be followed by PEI/DNA complexes. The high transfection efficiency of PEI *in vitro* has been ascribed to its ability to act as a proton sponge that buffers the low pH in the endolysosomal compartments and potentially induces ruptures of the endolysosomal membrane, resulting in the release of PEI/DNA complex into the cytoplasma (Boussif *et al*., 1995). It is usually branched with every third amino nitrogen atom being protonated such that PLL has a buffer capacity virtually at any pH value (proton sponge) (Kichler *et al*., 2001). This feature and PEI ability to destabilize lysosomal membranes enables PEI polyplexes to efficiently escape the degradation within the acidic endosomal environment (Kichler *et al*., 2001; Klemm *et al*., 1998).

Furthermore, Lechardeur *et al*. (1999) have shown that upon entry into the cytoplasm, naked plasmid DNA (pDNA) undergoes a rapid turnover because of degradation by cytosolic nucleases. Moret *et al*. (2001) have shown that PEI is able to protect pDNA against degradation by sernm DNases. Although the proton-sponge effect and the ability to deliver DNA to the nucleus enhance transgene expression, the presence of PEI in the cellular nucleus may interfere with transcriptional and translational processes and even induce cell death (Godbey *et al*., 2001). Efforts have been undertaken to diminish the high cationic charge density of PEI to a magnitude that promotes DNA delivery but decreases the adverse effects of PEI on cell viability (Putnam *et al*., 2001; Bogdan *et al*., 2002).

PEI/DNA/plasmid and Other Conjugate and Complexes

a) Plasmid and PEI Complex

In case of gene delivery to adult neural stem cells plasmids can be delivered *in vivo* when complexed with linear polyethylenimine and gene expression can be targeted specifically to neural stem or progenitor cells by the use of specific promotors. These techniques may be utilize both to study the function of various genes in the differentiation of neural stem cells to specific cell fates and ultimately for gene therapy or to generate specific differentiated progeny for cell transplantation (Falk *et al*., 2002).

b) Galactosylated PEI

Galactosylated PEIs (Gal-PEI) could be use for gene transfer. To optimize a receptor mediated and cell selective gene transfer with polyethylenimine (PEI) based vector three galactosylated PEIs (Gal-PEI) with different molecular weight were tested on asialglycoprotein receptor-positive cells (Morimoto *et al*., 2003).

c) PEI Conjugated to Antibody

Tumor-targeted gene delivery is performed via anti-HER2 antibody (trastuzumab, Herceptin) conjugated polyethylenimine. Vectors were DNA polycation complexes (polyplexes) prepared by mixing, at vector ratios, plasmid DNA carring a luciferase reporter gene to Her PEI, which is a conjugate of linear polyethylenimine(PEI), a cationic polymer and trastuzumab (Herectin), a HER2-specific monoclonal antibody. HerPEI polyplexes has shown promising HER2 receptor specific gene transfer properties and warrant further evaluation as a tumor-targeted vector for gene therapy (Chiu ***et al***., 2004).

d) PEI conjugated to Cholesterol

Non-viral polymer/plasmid DNA complexes were formed using linear polyethylenimine (LPEI) Mw 25k conjugated to cholesterol in a T-shaped geometry (LPC-T) and pDNA encoding murine interleukin-12 (pmlL-12e). These complexes were subsequently injected weekly into mice (BALB/C) intravenously and locally for the treatment of murine renal cell adenocarcinoma (Renca) induced pulmonary metastases and subcutaneous (SC) Renca tumors respectively. Thus it was concluded that LPC-T is an effective carrier for passive targeting of the pulmonary tissue, treatment of Renca-induced pulmonary metastases and local administration of Renca cell SC tumors (Furgeson ***et al***., 2004).

e) Cetyl PEI and DNA Complexes

A novel gene transfer system utilizing polycation liposomes (PCLs), obtained by modifying liposomes with cetylpolyethylenimine (PEI), is found. PCLs show notable transfection efficiency with low cytotoxicity. PCLs effectively transfer DNA to endosomes and release cetyl PEI-DNA complexes into the cytosol. Furthermore, cetyl PEI also contributes to gene entry into the nucleus (Sugiyama ***et al***., 2004).

Another report describes gene transfer *in vitro* as well as *in vivo* using cetylated low-molecular mass (600 Da) polyethylenimine (28% of amine groups substituted with cetyl moieties), termed CT-PEI. This compound is hydrophobic and has to be incorporated into liposomes in order to be suitable for gene transfer studies. Serum-induced plasmid DNA degradation assay demonstrated that CT-PEI-containing liposomal carriers could protect complexed DNA (probably *via* condensation). *In vitro* luciferase gene expression achieved using medium supplemented with 10% serum was comparable to that achieved in serum-reduced medium and was highest for CT-PEI/cholesterol liposomes, followed by CT-PEI/dioleoylphosphatidylcholine liposomes and PEI 600 Da (uncetylated) carrier. *In vivo* systemic transfer into mice was most efficient when liposome formulations contained CT-PEI and cholesterol. Higher luciferase expression was then observed in lungs than in liver. In conclusion: liposomes containing cetylated polyethylenimine and cholesterol are a suitable vehicle for investigating systemic plasmid DNA transfer into lungs (Aleksander ***et al***., 2004).

Factors That Influence the Improvement of Efficiency and Actions

Many factors affect the efficiency profile of PEI polyplexes (and almost any non-viral vector) such as molecular weight, degree of branching, ionic strength of the solution, zeta potential and particle size (Kunath ***et al***., 2003; Kircheis ***et al***., 1999). One study, for instance, showed that low molecular weight (10 kDa), moderately branched polymer resulted in efficient delivery with low toxicity in comparison with commercial high molecular weight PEI (Fischer ***et al***., 1999). Another study demonstrated that linear PEI (22 kDa) was more efficient in both salt and salt-free buffers than branched polymers (25 and 800 kDa) (Wightman ***et al***., 2001). Lethal side effects in mice, however, were observed when the linear PEI (22 kDa) polyplex was injected intravenously within its therapeutic window (Chollet ***et al***., 2002). Therefore, more studies are needed to produce optimum PEI carriers with respect to efficiency/toxicity behavior.

(PEI) Recent progress in consequent rational vector improvement is highlighted by our finding of polyethylenimine derivatives more potent and yet less cytotoxic than the 25-kDa polyethylenimine (one of the most effective non-viral vector). Such vectors could be further modified with cell targeting ligands to enhance their utility for *in vivo* applications (Thomas *et al*., 2003).

The nature of these polymers enables the researchers of successfully introducing targeting ligands and/or polyethylene glycol (PEG) (that produces sterically stabilized gene carriers) to their surfaces. Pegylated PEI polyplexes, for instance, were linked to tumor specific ligand transferrin, an asialoglycoprotein and then applied intravenously, resulting in five-fold increase in the transfection efficiency with lower toxicity in comparison with pegylated (transferrin-free) PEI polyplexes (Kircheis *et al*., 1999).

Stability Improvement

In vitro instability of a polycationic vector limits it's efficacy after systemic administration. Conjugation of hydrophilic polymers with neural charge onto polycationic vectors has been used to improve the stability by reducing the interaction between the vectors and the blood components, such as serum albumin. In an experiment, dextrans of molecular weight 10000 (dex-10000) and 1500 (dex-1500) were used to produce various degrees of grafting on linear and branched polyethylenimines (PEI) and the dextran-grafted polymers were used to prepare DNA-polymer complexes. The changes in size and in zeta potential and the extent of DNA release after the exposure of the complexes to bovine serum albumin (BSA) were used to evaluate the stability of the complexes prepared at various ratios of DNA to polymer. Finally it was found that the dextran-grafted branched PEI improved the stability of the DNA-polymer complexes and showed potential to conjugate with ligands for *in vivo* targeted gene delivery (Tseng and Jong, 2003).

Chitosan as Gene Delivery Vector

Chitosan, naturally occurring cationic polysaccharides, has been shown to excel in transcellular transport (Guang *et al*., 2002). It is a candidate nonviral vector for gene delivery because of it's high positive charges and low cytotoxicity. Uulike high molecular weight chitosan (HMWC), low molecular weight chitosan (LMWC) is highly water soluble and can form complex with plasmids in physiological buffer. The plasmid DNA was completely retarded at a weight ratio of 1:2 (plasmid : LMWC) in 1% agarose gel. DNase1 protection assay showed that plasmids were protected from DNase1 over 60 min. The most efficient transfection was obtained at a weight ratio of 1:3 (plasmid: LMWC). The transfection efficiency of LMWC was significantly higher than poly-L-lysine (PLL). MTT assay showed that LMWC was less cytotoxic than PLL. Therefore, LMWC will be useful in the development of safe gene carriers (Lee *et al*., 2001). The formulations with high molecular weight (HMW) chitosan can be an effective nonviral method of gene vector in animal studies. Two different preparation methods (the solvent evaporation method and the complex coacervation method) an the encapsulation of a model plasmid with chitosan was performed. The ability of different molecular weights of chitosan to form nanoparticles with a plasmid and particulate polymers to stabilize a plasmid in a supercoiled form, were examined by agarose gel electrophoresis. The efficiency of nanoparticles mediated transformation to *Escherichia coli.* cells was significantly higher than naked DNA or poly-L-lysine(PLL)-DNA polycation complexes. The transfection studies were performed in cos7 cells. A three fold increase in gene expression was produced by nanoparticles as compared to the same amount of naked plasmid DNA (Bozkir *et al*., 2004).

Chitosan also presents some charateristics favourable for gene delivery, such as the ability to condanse DNA and form small discrete particles in defined conditions. In controlled conditions, when plasmid DNA formulated with chitosan, produce homologous populations of complexes which were stable and had a diameter of approximately 50-100 nm. Discrete particles of nicely condensed DNA

had a donut, rod or even pretzel shape. Chitosan/DNA complexes efficiently transfected HeLa cells, independently of the presence of 10% serum and did not require an added endosomolytic agent. In addition gene expression generally increased over time, from 24 to 96 h whereas, in the same conditions the efficiency of polyethylenimine mediated transfection dropped by two orders of magnitude. At 96 h, chitosan was found to be 10 times more efficient than PEI. However, chitosan mediated transfection depended on cell type (Erbacher *et al*., 1998).

Poly (D, L-lactide-co-4-hydroxy-L-proline) (PLHP)

A new polymeric gene carrier, poly (D, L-lactide-co-4-hydroxy-L-proline) (PLHP) was synthesized and characterized to prove non-toxic. PLHP is a promising candidate for long term gene delivery with good biocompatibility and biodegradability while gene expression mediated by a non-viral vector usually lasts only a few days (Li and Huang, 2004). Degradation of PLHP was examined by changing the pH of the medium and molecular weight (MW) of the remaining polymer. The polymer was characterized by (1) Nuclear Magnetic Resouance (NMR) and Gel Permeation Chromatography (GPC). The cytotoxicity of PLHP is significantly lower than polyethylenimine (PEI) and poly-L-lysine hydrochloride (PLL). When attached with plasmid DNA gene transfer efficiency of PLHP/pDNA delivery system showed a sustained activity (over a week) when compared with PEI and PLL. It can be further improved by the addition of cationic liposomes.

Network Type Poly Amino Ester (n-PAE)

Efficient transfection and nontoxicity are two of the most important requirements of an ideal gene delivery vector. Artificially constructed polymeric vectors should have simultaneous multiple functions, i.e., controlled degradation and endosome disruptive function and positive changes. Conceding all these functions a network type poly amino ester (n-PAE) is found more suitable than PEI, which is one of the most effective polymeric gene delivery vectors, reported to date. n-PAE biodegradation and successive events of effective endosome escape result in high transfection efficiency and favorable cell viability response. The n-PAE mediated transfection is also very effective on the presence of serum (Lim *et al*., 2002).

Polycation/DNA Complexes

Self-assembling polycation/DNA complexes represent a promising synthetic vector for gene delivery. However, despite considerable versatility and transfectional activity *in vitro*, such materials are quickly eliminated from the blood stream following intravenous injection (plasma alpha half-life typically less than 5 min). For targeted systemic delivery a more prolonged plasma circulation of the vector is essential. It is found that rapid plasma elimination of polycation/DNA complexes results from their binding serum albumin and other proteins, perhaps due to aggregation and phagocytic capture or accumulation of the ternary complexes in fine capillary beds (Dash *et al*., 1999).

Adenovirus (Ad)/polycation Complexes

Complexing recombinant adenovirus (Ad) vector with various polycations has been shown to enhance transduction of cell lines. The activity of Ad/polycation complexes was tested *in vivo* in the mouse lung. Several polycations were capable of enhancing transduction of mouse respiratory epithelium, leading to a 1-2 log increase in levels of transgene expression. Poly-L-lysine (PLL) and DEAE-dextran were found to increase Ad-mediated gene transfer without any additional toxicity as assessed histologically or through the measurement of inflammatory cytokines in bronchoalveolar lavages. So, complexing Ad vectors with polycations has the potential to improve the therapeutic index by increasing transgene expression while reducing unwanted responses associated with high doses of vector (Kaplan *et al*., 1998).

Folate Targeted Transfection Complex

A folate targeted transfection complex that is internalized by certain cancer cells and displays several properties reminiscent of enveloped viruses has been developed. These liposomal vectors are comprised of a polycation condensed DNA plasmid associated with a mixture of neutral and anionic lipids supplemented with folate polyethyleneglycol dioleylphatidylethanolamine for tumor cell-specific targeting N-Citraconyl- dioleylphosphatidylethanolamine is also included for pH dependent release of endosome-entrapped DNA into the cytoplasm and a novel plasmid containing a 366 bp segment from SV40 DNA has been employed to facilitate transport of the plasmid into the nucleus. It was found that complexation of plasmid DNA with high molecular weight polymers such as acylated polylysine and cationic dendrimers leads to higher folate mediated transfection efficiency than DNA complexed with unmodified polylysine. In contrast, compaction of plasmid DNA with small cationic molecules such as spermine, spermidine or gramicidin S yields ouly weakly active folate-targeted liposomal vectors (Reddy *et al*., 1999).

Barriers of Using Cations in non Viral Gene Therapy

Extracellular and Intracellular Barriers

Complexes of DNA with cationic lipids and cationic polymers are frequently used for gene transfer. Extracellular interactions of the complexes with anionic glycosaminoglycans (GAGs) may interfere with gene transfer. Interactions of GAGs with carrier DNA complexes have been studied using tests for DNA relaxation (ethidium bromide intercalation), DNA release (electrophoresis) and transfection (pCMVbGal transfer into RAA smooth muscle cells). Several cationic lipid formulations (DOTAP, DOTAP/Chol, DOTAP/DOPE, DOTMA/DOPE, DOGS) and cationic polymers (fractured dendrimer, polyethylene imines 25 and 800 kDa, polylysines 20 and 200 kDa) were tested. Polycations condensed DNA more effectively than monovalent lipids. Hyaluronic acid did not release or relax DNA in any complex, but it inhibited transfection by some polyvalent systems (PEI, dendrimers, DOGS). Gene transfer by other carriers was not affected by hyaluronic acid. Sulfated GAGs (heparan sulfate, chondroitin sulfates B and C) completely blocked transfection, except in the case of liposomes with DOPE. Sulfated GAGs relaxed and released DNA from some complexes, but these events were not prerequisites for the inhibition of transfection. Furthermore, preliminary results suggest that cell surface GAGs, particularly heparan sulfate, inhibit gene transfer by cationic lipids and polymers (Ruponen *et al*., 2003).

The additional hydrogen bonding or covalent interactions of the headgroup with the plasmid DNA, leading to higher binding affinity of the cationic lipids to pDNA, results in higher transfection. This hypothesis is supported by TEM observations where elongated complexes were observed and more lipid was seen associated with the DNA (Narang *et al*., 2005).

Effect of Human Plasma Proteins

In situ gene expression assay indicated that both the ratio of DNA and liposome and the dose of DNA could affect the gene transfection efficiency. Naked endostatin plasmid intratumoral injection can get a similar gene transfection efficiency to liposome-DNA complex when used *in situ* (Ma *et al*., 2004).

Interaction of cationic lipid/DNA complex with the plasma is a limiting step for the cationic lipid-mediated intravenous gene transfer and expression process. Most of the plasma components that interact with the complex and inhibit its transfection efficiency are still unknown. In an experiment, human plasma proteins and lipoproteins that bind to a cationic lipid/DNA complex were isolated on a sucrose density gradient and identified by 2-D gel electrophoresis. Protein binding did not result in complex dissociation or DNA degradation. The effects of several complex-binding plasma components on the transfection efficiency were studied using lung endothelial cells cultured *in vitro*. Lipoprotein

particles caused a drastic loss of the transfection efficiency of the complex. Surprisingly, fibrinogen was found to activate the transfection process. The roles of these complex-binding plasma components on the complex uptake efficiency were quantitatively assessed using radiolabeled plasmid DNA and qualitatively evaluated using fluorescence microscopy. A good correlation was found between the effects of the complex-binding plasma components on the transfection and on cell uptake efficiencies. In contrast to what was generally believed, our data suggest that disruption of the complex does not occur when it is in contact with the plasma and therefore could not be responsible for the loss of transfection activity. Instead, coating of complexes with plasma components seems to be responsible for reduced uptake by cells, which in turn results in reduced transfection (Tandia *et al*., 2003).

Improvement of Transfection Efficiency

Efficiency Increasing by Surface Coating

Systemic gene delivery systems are needed for therapeutic applications. For systemic circulation, masking the surface charge of DNA complexes has to be accomplished to avoid interactions with plasma components, erythrocytes and the reticuloendothelial system. Polyplexes based on polyethylenimine (PEI), shielded with polyethylene glycol (PGE) and linked to the receptor binding ligands transferrin (Tf) or epidermal growth factor (EGF) have been developed. Complexes were found to mediate efficient gene transfer into tumor cell lines in a receptor dependent and cell-cycle dependent manner. Systemic administration of surface shielded Tf-PEI polyplexes into the tail vein of mice resulted in preferential gene into distantly growing subcutaneous tumors. In contrast, application of positively charged PEI polyplexes directed gene transfer primarily to the lung (Ogris and Wagner, 2002).

Delivery Efficiency and Gene Expression Efficiency Increasing Action

Tumor targeting is an important issue in cancer gene therapy. A gene transfection method, based on light inducible photochemical internalization (PCI) of a transgene, is already developed to improve gene delivery and expression selectively in illuminated areas, for example in tumors. PCI improve the non-viral vector polyethylenimine (PEI)-mediated transfection of therapeutic gene, the suicide gene encoding herpes simplex virus thymidine kinase (HSV+K). Photochemical transfection allows selective enhancement in gene expression and gene mediated biological effects (cell killing by the HSV+K/GCV approach) in response to illumination (Prasmickaite *et al*., 2004).

Systemic gene therapy vectors must be designed to safety and efficiently escort DNA from outside the cell to the nucleus and to overcome several physiological barriers that are obstacles to internalization, escape from endocytic vecicles, movement through the cytoplasm and transport into the nucleus. Chloroquine appears to facilitate PLL-mediated gene delivery by a mechanism other than buffering of endocytic compartments. Additionally, PEI does not appear to buffer endocytic compartments but requires exposure to an acidic enviroument for efficient gene delivery (Forrest and Pack, 2002).

Conclusion

Gene delivery/therapy is one of the youngest and most promising fields of medicine. In the course of one decade the basic research in recombinant DNA has been translated into a number of applied projects aiming at curing the diseases at its root, the gene. Though no clinically acceptable protocols have yet been developed, the researcher's ability to solve many technical problems of gene therapy has been greatly improved.

Several viral and nonviral gene therapy vectors have been evaluated for a range of conditions in animal models and in clinic. Cationic polymers and lipids showed a great potential among the vector implied for gene therapy and it also proved that they are safer than viral vectors. Many types of cationic polymers and lipids are tested but it is still a vital field of research to invent new one.

From this review, it has emerged that a single vector is highly unlikely to be optimal for all gene therapy applications. To improve gene transfer efficiency, the appropriate vector must be selected for transfection of the required cell type. Ultimately all disease targets will benefit from an improved understanding of the biology of vector delivery, uptake and expression. Still the perfect vector covering all therapeutic and safety requirements does not exist and much work is needed in this important research field.

Improvements in vector design will also reap rewards in the clinic. Research into vector design and targeting needs to proceed in parallel with early clinical studies, where proof of concept and identification of barriers to clinical gene transfer can inform the next step to basic research.

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