AN EMPHERICAL APPROACH IN FINDING THE DEVELOPMENT OF CATIONIC POLYMERS FOR NON-VIRAL GENE DELIVERY SYSTEM

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ABSTRACT:

Gene therapy is the process of treating a particular disease through the introduction ofgenetic material in order to elicit a therapeutic benefit. Initially, it was viewed as an approach for treatinghereditary diseases, but now wide recognition of its potential role in the treatment of acquired diseases such as cancer is being envisaged. Non-viral vectors based on the use of cationic lipids or polymers appear to have promising potential, given the problems of safety encountered with viral vectors.These vectors can be divided into two main categories, viral and non-viral systems. For the work performed here, the focus was on improving the efficacy of non-viral vectors (made of cationic polymers) in terms of improving transgene expression and reducing cytotoxicity in both in vitro and in vivo systems.When cationic polymers are mixed with plasmids (pDNA), they form polyp lexes which protect pDNA from hash environments and deliver pDNA to target cells.Our research goal was to develop polyplex formulations made from cationic polymers that generate high transfection efficiencies and low toxicities.

Keywords: *cationic polymers, Non-viral Vectors, Gene therapy, Transfection.*

INTRODUCTON:

Gene therapy is considered as an alternative for enzyme /protein replacement therapy. The disadvantages like in vivo clearance and manufacturing cost faced by the replacement therapy makes gene therapy a potential alternative for various rare genetic disorders.Gene therapy is defined as the procedure used to treat or improve the health condition of the patient by modifying the patient's cells genetically. It provides an unique approach to treat both inherited and acquired diseases by delivering a therapeutic gene material and its associated regulatory elements into the nucleus; in order to correct the loss of function caused by mutation or to express the deficient gene product at physiologic levels The delivery of functional genes to target cells for achieving therapeutic effect is defined as gene therapy. Gene therapy being treatment or prevention of disease by gene transfer is being considered as a potential medical revolution. However, the biological system being complex becomes an obstacle for successful gene delivery.This process of DNA delivery into cells is known as "transfection" and it is important that the efficiency of transfection be optimized such that a patient can obtain maximum therapeutic benefit from such a treatment. DNA is susceptible to being destroyed by harsh physiological environments prior to reaching its target. The development of effective polyplex formulations requires optimization.

Among the cationic polymers being used as non-viral vectors, PEI has been considered the gold standard for transfection in vitro and in vivo and is a promising alternative carrier to viral vectors. The factors that contribute to the ability of PEI complexes to mediate efficient transfection include: enhanced protection of DNA from enzymatic degradation, enhanced internalization by cells, and the ability to mediate endosomal escape and then release the DNA.

DIFFERENT METHODS OF GENE THERAPY

Germline gene therapy: The technology of this type of gene therapy is simple as genetic abnormalities can be corrected by direct manipulation of germline cells with no targeting, and not only achieve a cure for the individual treated, but some gametes could also carry the corrected genotype. Although it almost never has been tested on humans, some different transgenic techniques have been used on other species, which include the following:

(1) Gene delivery to the nuclei taken from somatic cells at metaphase stage.

(2) Ex vivo alteration of egg cells, following in vitro fertilization.

(3) Manipulation of embryonic stem cells of mouse during in vitro culture by different gene delivery systems.

(4) Pronuclear microinjection of exogenous DNA solution by a glass needle.

(5) Transgenic delivery into sperm cells by direct or indirect injection to testis or other parts of the genital system.

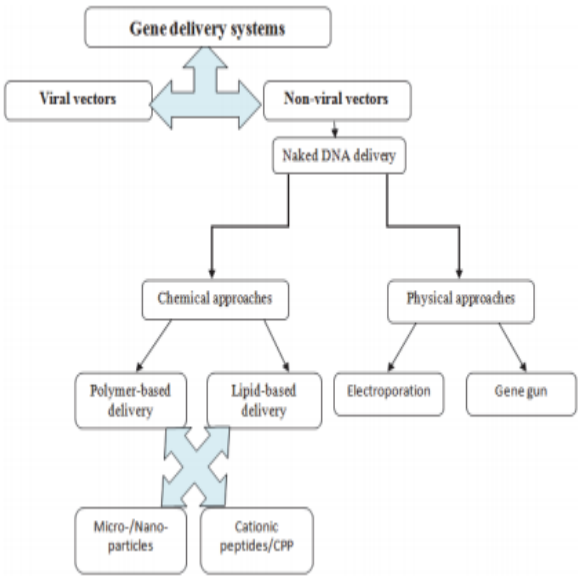


Figure 1: Different gene delivery systems

Somatic gene therapy:

Somatic gene therapy involves the insertion of genes into diploid cells of an individual where the genetic material is not passed on to its progeny. Somatic cell therapy is viewed as a more conservative, safer approach because it affects only the targeted cells in the patient, and is not passed on to future generations; however, somatic cell therapy is short−lived because the cells of most tissues ultimately die and are replaced by new cells. In addition, transporting the gene to the target cells or tissue is also problematic. Regardless of these difficulties, however, somatic cell gene therapy is appropriate and acceptable for many disorders.

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 mainly through the endocytosis pathway.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. Non viral gene transfer vectors have been actively studied in the past years in order to obtain structural entities with minimum size and defined shape.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, galactosylatedssDNA 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 .

Cationic Polymers:

Cationic polymers are usually classified in two main groups: natural polymers, such as proteins and peptides, polysaccarides, and synthetic polymers, such as polyethyleneimine (PEI), dendrimers, and polyphosphoesters. They interact with the DNA through electrostatic interaction by means of amines and/ or ammonium ions. The ratio between the number of vector's amines and the number of phosphates in the pDNA is referred to as the N/P ratio, which dictates morphology and size of the complex. Among natural polymers, the cationic polysaccharide chitosan has been probably one of the most widely studied non-viral vectors with numerous published trials, both in vitro and in vivo. Chitosan is nontoxic even at high concentrations and at all molecular weight ranges. However, although it shows effective nucleic acid binding and compaction, the delivery efficiency is generally low in most cell lines. Nevertheless, due to its mucoadhesive properties, chitosan/DNA polyplexes have been successfully applied to oral and nasal gene therapy. The latest strategies to improve its transfection ability comprise the synthesis of novel chitosan derivatives, such asminoethyl- chitin (ABC) ,thiolated- chitosan , chitosan methoxy poly-ethylene glycol- cholesterol (LCP- Ch) and low molecular weight alkylated chitosans. To overcome intracellular barriers, such as crossing the cell membrane, recently chitosan was conjugated to folic acid (FA) for targeting tumor cells allowing folate- receptor mediated endocytosis.

Recently, more polymers with improved biocompatibility and biodegradability have been reported demonstrating equal or superior performance comparing to nondegradable PEIs. Among these are aminoesters or oligoamines polymerized through disulfide linkages or polyamino acid derivatives with proton absorption capacities. Besides PEI and more recent polyamines of varied structures, synthetic or natural polypeptides and their derivatives have been explored as gene delivery vehicles. These include poly(l-lysine) (PLL), polyornithine, polyarginine, histones, and protamines that have excellent ability to condense DNA.

Polymer	Abbreviation	Unique feature
Poly(ethylene)glycol	PEG	Inert
Polyethylenimine	PEI	Cationic
Dithiobis(succinimidylpropionate)	DSP	Biodegradable PEI
Dimethyl-3,3′-dithiobispropionimidate	DTBP	Biodegradable PEI
Poly(ethylene imine) biscarbamate	PEIC	Biodegradable PEI
Poly(L-lysine)	PLL	Cationic
Histidine modified PLL		Biodegradable
Poly(*N*-vinylpyrrolidone)	PVP	Neutral
Poly(propylenimine)	PPI	Dendromer
Poly(amidoamine)	PAMAM	Dendromer
Poly(amido ethylenimine)	SS-PAEI	Biodegradable
Triethylenetetramine	TETA	Cationic
Poly(β-aminoester)		Biodegradable
Poly(4-hydroxy-L-proline ester)	PHP	Biodegradable
Poly(allylamine)		Cationic
Poly(α-[4-aminobutyl]-L-glycolic acid)	PAGA	Biodegradable
Poly(D,L-lactic-co-glycolic acid)	PLGA	Biodegradable

Table 1: Polymers Commonly Used for Gene Transfer

Technical challenges and limitations to successful Non-Viral Gene transfer

The major technical limitations or critical steps in attaining a successful gene therapy are categorized into efficiency of vector transport and unloading into target cells,

perseverance, activity, immune response, regulatory issues and ethical concerns and commercialization. These different stages pose a big challenge to gene therapy to be efficiently treating the disease. The cost of gene therapy creates an image that it is meant for the affluent. This was clearly evident with the first commercialized gene therapy Aliopogenetiparvovec for Lipoprotein Lipase deficiency. The estimated treatment cost for LPLD gene therapy is about 1.6million/patient. This tends to be the major hurdle in commercializing the gene therapy if proven successful.

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.

Chitosan as Gene Delivery Vector

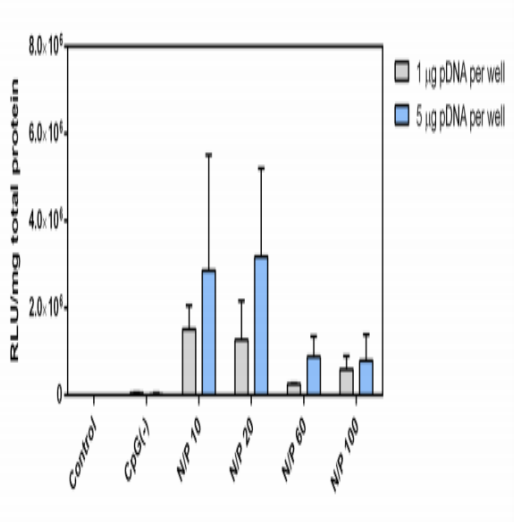
Chitosan, naturally occurring cationic polysaccharides, has been shown to excel in transcellular transport. It is a candidate nonviral vector for gene delivery because of it's high positive charges and low cytotoxicity. Unlike high molecular weight chitosan (HMWC), low molecular weight chitosan (LMWC) 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).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.

DISCUSSION

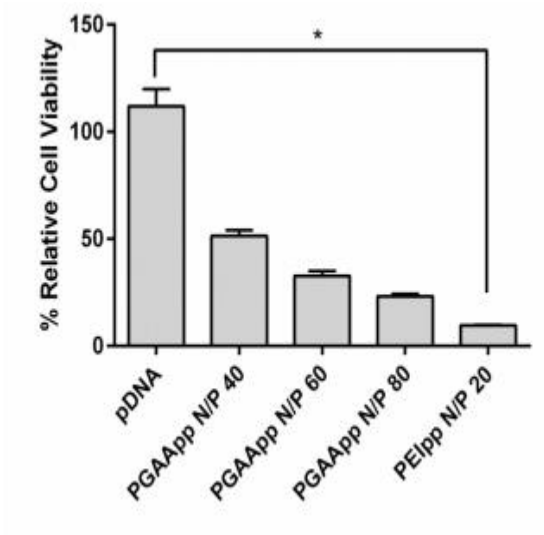
Cationic polymers shown promise as a predictable safe biodegradable alternative to virus, but the problem is its unpredictable endocytosis. The other major issue is its cytotoxicity. Coating with human serum albumin, dextran, PEG is considered. Theoretically this step demonstrated as less cytotoxic, but in vivo exhibited immune response. Chitosan being ecologically safe and of low toxicity and immunogenicity has been studied for almost two decades. Still the solubility (insoluble under physiologic pH) remains as main limitation. To improve this limitation hydrophobic and hydrophilic modifications are considered this include such as deoxycholic acid modification, thiolation, PEGylation, quarternization. Nevertheless ideal transfection efficiency was not attained due to certain factors acted differently.

Traditional non-viral vectors like various lipoplexes and polyplexes (polyethylenimine)

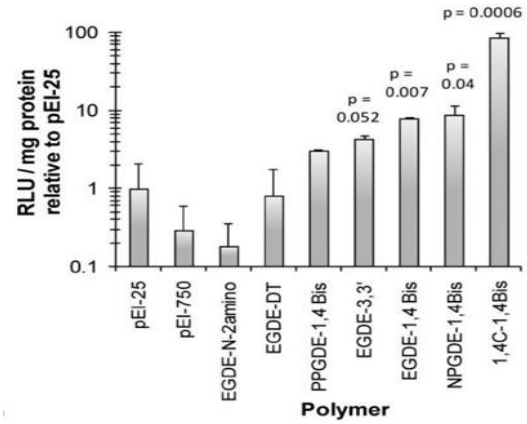
showed excellent results in invitro experiments, but their translation to in vivo is not effective and able to confer only transient gene expression. Nevertheless endosomal escape remains a critical bottleneck for non-viral vectors. Finally the last hurdle is not able to replicate in the nucleus and lost during mitosis.Presently researchers are concentrating more on developing cell penetrating peptides, nano shell, sleeping beauty transposon, conjugated polymers, and biological vectors to be effective in non-viral gene transfer as compare to viral vectors. Apart from above mentioned SPION (super paramagnetic nano particle), mitochondria targeting strategies {mitochondria leader peptide (MLP), mitochondria targeting sequence (MTS) +DNA, liposome based carrier (Dequalinium) DQAsomes} are also under present review of developing into potential gene transfer agen.



Transfection efficiency of A549 cells treated with CSpp(CpG(-)) at two different pDNA concentrations (1 µg (grey) and 5 µg (blue) pDNA per well). Varying N/P ratios are indicated



Cytotoxicity of PGAApp in HEK293



Comaprison of transfection efficiency of lead polymers of a diglycidyl ether polyamine

CONCLUSION:

Gene therapy has a potential to treat some of the life threatening orphan diseases. Advances in Genome sequencing and geneticanalysis have improved our understanding of human diseases, diagnostic ability but therapeutic benefit remains largely ineffective. Failure of finding an ideal vector remains major hurdle in treating human diseases with gene therapy. Past few years the trend for using non-viral vectors is significantly increasing. Further improvements to increase the transfection efficiency are needed before to see any

remarkable clinical results. These achievements might be relied on our understanding of limiting steps and methods to overcome it. Although non-viral vectors may work reasonably well "in vitro", clinical success is still far from ideal. Considering the number of research groups that focus their investigations on the development of new vec- tors for gene therapy, together with the advances in the development of new technologies to better understand their "in vitro" and "in vivo" behavior, the present limitations of non-vi-ral vectors will be resolved rationally

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