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Review

Polyethylenimine: A versatile, multifunctional non-viral vector for nucleic acid delivery

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ABSTRACT

Polyethylenimine (PEI) has recently been widely studied for the design of nucleic acid delivery vehicles. Gene delivery using PEI involves condensation of DNA into compact particles, uptake into the cells, release from the endosomal compartment into the cytoplasm, and uptake of the DNA into the nucleus. PEIs being positively charged, linear or branched polymers are able to form nanoscale complexes with small RNAs, leading to RNA protection, cellular delivery, and intracellular release. This review highlights the important properties of various PEIs with regard to their use for nucleic acid delivery. Brief discussion on cellular uptake mechanism of non-viral vector is included to understand its utility for gene delivery. Applications of modified PEI for increased efficacy, altered pharmacokinetic properties; improved biocompatibility and targeted delivery have also been discussed. An overview of simulation studies which can help in understanding the underlying complexation mechanism has also been included. The review provides a brief discussion about clinical trials and patents related to nucleic acid delivery using PEI based systems.

Contents

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1. Introduction

The vast amount of research done at molecular level has revealed the molecular mechanisms of many inheritable or acquired diseases which rely on the disrupted up-regulation or down-regulation of certain genes led to the expansion of research in the field of gene therapy. The strategy involving controlling of such genes can therefore open up new avenues for treatment and therapeutic intervention of various diseases. Gene therapy is the most talked about technique of the modern time involving delivery of genetic materials inside cells for the treatment of variety of diseases such as cancers, viral infections, hereditary diseases, genetic disorders etc. to name few [1,2]. Even though gene therapy is the most recent and potential strategy for combating various diseases, the success of the same has been limited due to the lack of safe and efficient carrier systems. Viruses are the most widely studied delivery carriers for gene therapy but their use has been limited due to their safety concerns which paved the pathway for development of non-viral gene delivery systems [3]. The concept of non-viral gene transfer can be described simply as "the use of carriers other than virus which (a) mimics viral infection processes; (b) condenses DNA; (c) protects DNA from degradation; (d) promote cellular uptake and nuclear delivery; (e) are non-immunogenic and non-cytotoxic".

Several non-viral vectors involving lipids, polymers and peptides have been developed in the past many years for use as non-viral gene delivery carriers [4]. These polymers condense with nucleic acids and form nanoparticles to facilitate the gene delivery, with the advantages of low toxicity, cost effectiveness, ease of production, and versatility for different applications. Amongst the various non-viral vectors developed, cationic polymers have been considered as the most promising candidates with enormous potentials and advantages in comparison to their counterparts owing to their unique characteristics of forming polyelectrolyte complexes with genes and ability to protect them from various enzymes [4]. Cationic polymers, containing several amine groups in their backbones have been studied extensively as gene carriers owing to the interaction between the positively charged polymer backbone and negatively charged DNA which causes spontaneous formation of compact nano-sized polyplexes. The charge neutralized compact polyplex core protects the enclosed nucleic acids from nucleases and hence maintain their stability and integrity till cellular uptake of polyplexes takes place [5]. Amongst the various cationic polymers containing amine group in their backbone, polyethylenimine (PEI) is the most widely investigated cationic polymer for delivery of nucleic acids. The present review describes about the properties of PEI which makes it a suitable candidate as non-viral nucleic acid delivery vehicle. It briefly describes the mechanisms of gene delivery and cellular uptake and elaborates upon various structural modifications of PEI for enhancing its transfection efficiency. The review further deals with the clinical trials involving PEI based delivery systems, recent advancement in the field of PEI based delivery systems including patents and their probable application in the field of pharmaceutical research.

2. Cellular uptake pathways for non-viral vectors

A brief description of the cellular uptake pathways for non-viral vectors is provided to understand the utility of PEI as a carrier for gene delivery. Lot of research has been conducted to study the cellular uptake mechanism of DNA-polymer complexes (lipoplexes and polyplexes). It has been reported that polyplexes and lipoplexes demonstrate different cellular uptake mechanisms in cell lines (A549 pneumocytes and HeLa cells). Both these non-viral gene delivery vectors cannot cross the plasma membrane due to their hydrophilic nature and larger size respectively, and hence undergo endocytosis, which has been established as the main mechanism of internalization of non-viral vectors in cells [6–8]. Cellular uptake of lipoplexes proceeds only by clathrin-mediated endocytosis (CME), whereas polyplexes are taken up by two mechanisms — one involving caveolae (which has slower uptake kinetics) and another using clathrin-coated pits [9]. Reports suggest that particle size affects both clathrin and caveolae mediated endocytosis. The clathrin mediated pathway of endocytosis shows an upper size limit for internalization of approx. 200 nm, and kinetic parameters may determine the almost exclusive internalization of such particles along this pathway rather than via caveolae. Although the cellular uptake kinetics of caveolae mediated endocytosis is slower than CME, the polyplexes taken up via the caveolae escape the lysosomal compartment and hence have a high level of transfection efficiency. Such observations highlight the importance of studying the cellular uptake mechanism and transfection mechanism. The various cellular uptake pathways for non-viral gene vectors have been summarized in the present review (Figs. 1, 2).

2.1. Endocytic uptake pathway

Endocytosis is the cellular uptake of macromolecules and solutes into membrane-bound vesicles derived by the invagination and pinching off of the plasma membrane basically involving three modes viz. fluid-phase, adsorptive, and receptor-mediated endocytosis [10]. Fluid-phase has low-efficiency and is a non-specific process whereas, in adsorptive and receptor-mediated endocytosis, molecules preferentially interact with generic complementary binding sites or bind to receptors on the cell surface and become concentrated before internalization and hence have high efficiency of internalization.

In case of clathrin mediated endocytosis, the first step of internalization is the strong binding of a ligand to a specific cell surface receptor which results in clustering of the ligand-receptor complexes in coated pits on the plasma membrane. These are formed by the assembly of cytosolic coat proteins mainly clathrin and adaptor protein complexes, and mediate the assembly of the clathrin-lattice on the membrane [11,12]. The coated pits then form intracellular clathrin-coated vesicles. The PEI/DNA complexes entering via this pathway experience a drop in pH from neutral to pH 5.9 to 6.0 in the lumen of early endosomes, with a further reduction to pH 5 during progression from late endosomes to lysosomes. Transferrin can be used to promote clathrin mediated endocytosis but genes undergoing cellular uptake by this process might experience endosomal degradation.

Caveolae-Mediated Endocytosis involves cholesterol-binding proteins called caveolins, which function to create and/or mediate caveolae mediated endocytosis of DNA complexes. The mechanism of cellular uptake via this pathway was elucidated by visualizing the trafficking of the SV40 that uses caveolae to gain entry into the cells [13]. Caveolae uptake is a non-acidic and non-digestive route of internalization. The advantage of this pathway is that there is no drop in pH, which protects the molecule which can be directly transported to the Golgi and/or endoplasmic reticulum, thus avoiding normal lysosomal degradation [14]. Macropinocytosis involves formation of large endocytic vesicles of irregular size and shape, generated by actin-driven invagination of the plasma membrane. Owing to its large size, macropinocytosis is an efficient route for non-selective endocytosis of macromolecules. This route facilitates the bulk uptake of soluble antigens by immature

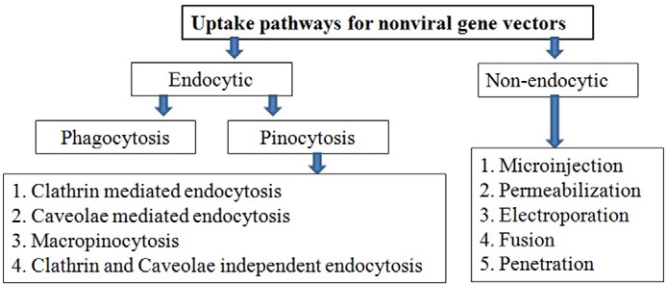

Fig. 1. Cellular uptake pathways for non-viral based gene delivery systems.