Poly(lysine) Dendrimers

Subjects: Pathology

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The disruption of the cellular pathways of protein biosynthesis through the mechanism of RNA interference has been recognized as a tool of great diagnostic and therapeutic significance. However, in order to fully exploit the potential of this phenomenon, the efficient and safe carriers, capable of overcoming extra- and intracellular barriers and delivering siRNA to the target cells are needed. Recently the attention has been paid to the possibility of application of multifunctional nanoparticles – dendrimers as potential delivery devices for siRNA. The aim of the present work was to evaluate the formation of dendriplexes using novel poly(lysine) dendrimers (containing lysine and arginine or histidine residues in their structure), and to verify the hypothesis that the use of these polymers may allow to obtain an efficient method of siRNA transfer into the cells in vitro. The fluorescence polarization studies, as well as zeta potential and hydrodynamic diameter measurements were used to characterize the dendrimer:siRNA complexes. The cytotoxicity of dendrimers and dendriplexes was evaluated with the resazurin-based assay. Using the flow cytometry technique, the efficiency of siRNA transport to the myeloid cells was determined. This approach allowed us to determine the properties and optimal molar ratios of dendrimer:siRNA complexes, as well as to demonstrate that poly(lysine) dendrimers may serve as efficient carriers of genetic material, being much more effective than the commercially available transfection agent – Lipofectamine 2000. This outcome provides the basis for further research on the application of poly(lysine) dendrimers as carriers for nucleic acids in the field of gene therapy.

poly(lysine) dendrimers siRNA gene delivery gene therapy transfection

1. Introduction

RNA interference (RNAi) is a biological process in which RNA molecules induce specific inhibition of target gene expression. Its discovery provoked great enthusiasm in the scientific community, and subsequent studies on RNAi resulted in the transition from experimental technology to a powerful therapeutic tool. In 2010, the first case of systemic targeted delivery of short interfering RNA (siRNA) was reported, which gave a solid foundation for the clinical use of this type of genetic material [1]. In recent years, siRNA has become the basis for drug development due to the high level of specificity, limited side effects, and the ease of synthesis of therapeutic molecules [2].

However, before siRNA molecules reach their site of action in the cell cytoplasm, they have to conquer several obstacles, the number of which depends largely on the way of administration, the target tissue, as well as the physicochemical properties of the siRNA itself. The latter involve low stability, negative charge, and high structural stiffness, hampering the transport in body fluids, across cellular membranes, and between intracellular

compartments [3]. What is more, siRNA upon entering the bloodstream easily undergoes enzymatic degradation by nucleases or rapid renal clearance [4].

In order to overcome the barriers limiting the transport of genetic material and exploit the therapeutic potential offered by the RNAi mechanism, effective delivery systems for siRNA molecules to their site of action are necessary. These systems, in addition to specific transport and transfection efficiency, should be able to protect siRNA against degradation by nucleolytic enzymes, prolong its blood circulation time, and release genetic material intracellularly, making it easily accessible for RNAi machinery, thus enabling effective silencing of the target gene [5].

In general, the carriers used to introduce nucleic acids into the cells can be divided into viral and non-viral systems. Vectors using genetically modified viruses are capable RNA delivery devices, offering, among others, long-term silencing of gene expression even after a single administration, efficient transfection, or expression of multiple copies of siRNA molecules. However, expensive production and side effects associated with random integration into the host genome (which can lead to the damage of important genes or activation of oncogenes), significant immunogenicity, and the possibility of the virus returning to its wild pathogenic type may limit the use of this group of carriers. Therefore, efforts have been made to obtain synthetic non-viral siRNA carriers, useful for both in vitro and in vivo applications [6][7].

Currently, the most commonly used non-viral siRNA carrier systems are based on nanomaterials. For this purpose, natural and synthetic cationic polymers are often applied [8], including highly branched dendrimers of a well-defined structure. These compounds enable electrostatic interactions with negatively charged siRNA molecules, providing the formation of stable complexes (dendriplexes) that are capable of intracellular delivery of nucleic acids and their protection against the activity of nucleases. What is more, surface groups of dendrimers may be subjected to modifications by functional moieties targeting them to specific locations. Numerous experiments have shown that dendrimers can be successfully used as carriers of various types of genetic material, including plasmids, single strands of DNA, oligonucleotides, and finally RNA molecules, ensuring their improved stability and prolonged blood half-life [9][10].

2. History and Development

Most studies on the potential use of dendrimers as carriers for siRNA concern poly(amidoamine) (PAMAM), poly(propyleneimine) (PPI), and poly(lysine) dendrimers (PLL) belonging to the group of peptide dendrimers [10]. The latter are branched polymeric structures in which both the core and dendrons are composed mainly of amino acids connected by peptide bonds [11]. Peptide dendrimers are most often based on lysine, an amino acid that enables the generation of several branching points [12]. PLL dendrimers, due to their flexible structure and protein-like characteristics, such as good biocompatibility and water solubility, as well as high resistance to proteolytic digestion could be used in several biomedical applications. Their properties have been studied over the last few years both experimentally [13][14][15] and in computer simulation [16][17][18][19][20][21]. In particular, they have aroused the interest of several research teams aiming at their use as carriers of nucleic acids [22][23][24][25].

The primary goal of this study was to investigate the formation of complexes of three types of PLL dendrimers (containing additional lysine, arginine, or histidine residues within their structure) with siRNA molecules, to assess the cytotoxicity of dendrimers themselves and the obtained dendriplexes, and finally to evaluate the efficacy of transfection in in vitro-cultured myeloid cell lines.

The performed in vitro tests allowed demonstration of the differences in the cytotoxicity of the tested PLL dendrimers, as well as the high biocompatibility of dendriplexes formed by this type of polymer. Most importantly, a very high level of cellular uptake of dendrimer:siRNA complexes was shown, exceeding 90% regardless of the dendrimer structure. These studies constitute the first stage of evaluation of the possibility of the application of the studied dendrimers as carriers for siRNA and will be continued in a wider scope. Only after demonstrating that the transported nucleic acid effectively inhibits expression of the target gene will it be possible to state that PLL dendrimers are an effective method of cell transfection, and may in the future become the basis of modern gene therapy. Additionally, considering the differences in the physicochemical properties of dendriplexes formed by various types of PLL dendrimers, it is reasonable to continue the studies in the field of their characterization, in order to elucidate the role of different amino acids in the structure of the dendrimer in the interactions with siRNA.

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