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Dendrimers as Delivery Systems in Gene Silencing Studies

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Abstract: RNA interference (RNAi) is a natural defence pathway in a variety of species, which leads to the posttranscriptional gene silencing - degradation of target messenger RNAs in a gene-dependent manner. This phenomenon has been employed to manipulate gene expression by introduction of small interfering RNA (siRNA) into the cell. Researchers are now developing RNAi-based interventions for the prevention and treatment of human diseases such as viral infection, tumors and metabolic disorders. However, RNAi-based drugs require usage of efficient carriers to permit the genetic material to cross the plasma membranes of target cells. In this review, we will define key terms of this breakthrough technology and mention some prerequisites of the RNAi-based therapy that must be fulfilled for this treatment to work. We will also point at some unique properties of dendrimers as carriers for targeting nucleic-acid materials and introduce carbosilane dendrimers that are currently being studied by our research team.

Keywords: Dendrimers, Carriers, RNA interference, Gene therapy, Biomembranes

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1. Introduction

Nano-sized particles exhibit unique chemical, biological, electrical and mechanical properties. Recently much attention has been devoted toward using nanotechnologies for medical application (nanomedicine), since there is a growing evidence that nanomedicine has the potential to cure diseases and repair tissues by manipulating individual cells at the molecular level. An experimental way of using nanotechnologies to treat or prevent disease is a gene therapy approach. The gene therapy is mostly aimed at replacing an abnormal disease-causing gene or inactivating a mutated gene that is not working properly (or at activating a gene that should work but it does not). The method requires using a carrier called a vector in order to bring the new gene into the body's cells [1].

2. RNA interference-based approach

RNA interference (RNAi)-based gene therapies have recently become promising [1 3]. RNAi is actually an adaptive defence mechanism that occurs naturally in some organisms and is triggered by the double-stranded RNA. The direct application of a sequence-specific, double-stranded RNA into an organism causes selective posttranscriptional gene silencing and thus the inability to produce the protein corresponding to the applied gene. Therefore, RNAi phenomenon has been widely investigated in order to silence expression of selected genes in mammalian and human cells. However, in mammals, it was observed that double-stranded RNAs longer than 30 nucleotides activate an antiviral response, which leads to the nonspecific degradation of messenger RNA (mRNA) transcripts, production of interferon, and overall shutdown of protein syntheses in the host cell [4]. But, gene specific suppression in mammalian cells can also be achieved by using in vitro synthesized short (small) interfering RNAs (siRNAs), 20 25 nucleotides in length. These synthesized siRNAs are long enough to stimulate gene-specific suppression but short enough to avoid the host interferon response [4]. Since optimisation of the use of RNAi in mammalian systems [2], the RNAi pathway has become a possible therapeutic strategy in the gene therapy of some diseases. The existing strategies can be broadly divided into two categories, namely, expressed short-hairpin RNAs introduced into cells using viral vectors, as well as delivered siRNA (termed also as a silencing RNA).

The use of viruses, though effective, results in immunogenic response, which limits its potential for clinical applications. Therefore, the majority of proposed clinical applications employ siRNA duplexes delivered into the cells by nonviral vectors to protect the host from viral infection [5]. After being delivered to the target, they are expected to inhibit the expression of viral antigen and accessory genes, control the transcription and replication of viral genome, hinder the assembly of viral particles, and influence the virus-host interactions.

However, despite several potential therapeutic applications, the efficiency of siRNA in vivo and in vitro is limited by its low resistance against enzymatic degradation, limited permeability across cell membrane, and substantial liver and renal clearance [6]. Therefore, alternatively, antisense oligo(deoxy)nucleotides (ODNs) may be used instead of siRNA. A comparison of different gene silencing strategies can be found at the pages of The National Center for Biotechnology Information [7]. Synthesized antisense ODNs, unlike siRNA, are single strands of RNA (or DNA) that are complementary to the chosen sequence of a gene that is known to be causative of a particular disorder. The oligonucleotide binds to the complementary mRNA sequence and inactivates the gene by preventing protein translation that will otherwise be produced by that particular gene. Antisense ODNs and siRNA duplexes both are polyanionic macromolecules that do not readily enter cells and typically require the use of a proper and applicable delivery system for effective gene silencing. Therefore, in order to exploit their potential therapeutic applications as well as to secure optimal bioavailability, and non-toxicity of the treatment to the individual, the effective way of delivery of siRNA/oligonucleotide into targeted cells and to the site of action has to be established first.

3. Dendrimers as potential carriers of nucleic material

One of the most common methods used for the delivery of charged nucleic acids involves their electrostatic interaction with cationic carriers. Several types of cationic carriers such as liposome/lipids, dendrimers [8], and polymeric amines have been successfully used for the delivery of plasmid DNA and ODNs. Because of their versatility and customizability, dendrimers belong to the most commonly studied nonviral vectors [9].

A unique characteristic of dendrimers is that they can act as a particulate system while retaining the properties of a polymer. They are a type of highly branched, monodispersive spherical macromolecules (their structure is shown in [10]) composed of a multifunctional core molecule with attached polymer branches [11]. Dendrimers are flexible in terms of their size, shape, branching, length, and peripheral groups that dominate the surface properties of the dendrimer in question. Dendrimers can be structured to encapsulate a drug or a diagnostic agent. Or the dense peripheral groups can be functionalized with various chemical agents bound to the surface of the dendrimer by noncovalent or covalent interactions which held until the changed conditions enable the agent to be released at the target site [11–13]. Thus, because of their properties and ability to be conjugated with a wide range of drugs, targeting moieties, and other biologically active components, dendrimers are intensely studied for applications in pharmaceutical and medical sciences [11, 12]. However, these highly efficient delivery systems have been less explored for nucleic-acid material delivery.

4. Dendrimers in the delivery of anti-HIV1 oligonucleotides

Recently, different kinds of dendrimers have been widely studied as efficient vehicles for the delivery of genes and therapeutic drugs [9, 11, 12, 14]. It has been shown that polyamidoamine (PAMAM) dendrimers or their conjugates are more efficient in delivering antisense ODNs or plasmid DNA than siRNA [14]. Cationic dendrimers with positive surface charge interact with negatively charged cell membranes and permeate more easily than the neutral or anionic dendrimers. However, the positive surface charge renders cytotoxicity to the cationic dendrimers, which imposes a serious limitation on the potential medical use. The transfection efficiency increases as a function of the dendrimer generation too. Good results are typically achieved with the sixth or seventh generations of PAMAM dendrimers [15], between the third and fifth generations of phosphorus-containing dendrimers [16]. Other kinds of dendritic macromolecules, such as polypropylenimine (PPI) [17] and poly(lysine) [18] dendrimers, have also been studied as potential DNA or ODN carriers. For instance, low generation PPI dendrimers have also shown gene transfection ability in vitro with low cytotoxicity. Although, at higher generations, increased toxicity prohibits their use.

Since HIV/AIDS continues to be a major public health problem worldwide with millions of people currently infected, in the last few years many anti-viral drugs have been developed to fight and control HIV infection. However, many phase II and phase III trials have reported severe side effects (i.e., liver toxicity), but particularly emergence of drug-resistant strains. The most classical example is GEM-91 – a phosphorothioate antisense oligonucleotide complementary in sequence to gag mRNA of HIV that

reached phase III clinical trial, but it was interrupted due to secondary effects [19]. The problem is the tendency of antisense drugs to the non-specific binding to serum proteins, because of their anionic charge. Those interactions lead to the decrease of antisense-drug bioavailability. To achieve desired therapeutic effects, the higher doses of drug are necessary, which on the other hand can induce toxic effects. We have already shown that formation of dendriplexes (dendrimer- ODN complexes) protected the anti-HIV1 ODNs (SREV, ANTI TAR, GEM91) from binding to bovine serum albumin (BSA) [20, 21]. HIV virus contains some genes in its genome which can be a potential target for siRNA. Therefore, there is much hope for efficient AIDS therapy with the use of RNAi. Every drug based on RNAi should be stable, selective, non-toxic and permeating membranes easily. Thus, a lot of effort is put to find a proper delivery vehicle for siRNA targeted against viral mRNA.

One of the major limitations for the use of siRNA, both in vivo and in vitro, is inability of naked siRNA to passively diffuse through the cell membranes due to its strong anionic charge and electrostatic repulsion from the anionic membranes [22]. Therefore, the RNAi therapy requires using the efficient carriers to permit the genetic material to cross biological membranes. Our team has started research of water-soluble carbosilane-based dendrimers as potential carriers for DNA or ODNs. These dendrimers are not available commercially and have been synthesized at the University of Alcala de Henares, Madrid, Spain. These dendrimers have shown a good transfection ability and low cytotoxicity [20, 21, 23]. The mechanisms of interaction of dendrimers with biomembranes are not known in sufficient details yet. Based on the results of our previous works focused on the study of the mechanisms of interaction of various ligands with biological membranes [24–26] and lipid bilayers [27–29], including ODNs [30–32] and dendrimers as biosensors [33], we believe that with the use of additional experimental techniques, such as dynamic light scattering, Langmuir-Blodgett method, etc., we will obtain important information on the mechanisms of dendrimer-membrane interactions. The primary focus will be on dendrimers as potential non-viral vectors for siRNA targeted against viral RNA of HIV-1 by studying the interactions of dendriplexes with model and biological membranes [10]. The second generation water-soluble carbosilane dendrimers with two types of terminal branches are being used: Si-O based branches and/or Si-C based branches both containing amino group terminations [10, 34]. Further the dendrimers will be complexed with siRNA derived from the viral structural gag gene (siGAG) to inhibit HIV replication [35].

5. Conclusion

Despite significant advances that have been made in gene silencing technologies, clinical applications are restricted with many factors such as bioavailability, stability, toxicity, cost, etc. First of all, the effective gene silencing requires using efficient carriers to permit the genetic material to cross the plasma membranes of the target cells. In this short review we have defined some key terms of this breakthrough technology, focused on some application requirements for dendrimers as carriers for targeting siRNAs and antisense ODNs, and outlined our research objectives. Our joint research work is aimed at understanding the interaction of both, cationic carbosilane dendrimers and their complexes with nucleic acids, with biological membrane structures. For this purpose we have currently been working

with model lipid monolayers and bilayers in order to describe the interactions of the dendrimer complexes with the membranes in terms of selected biophysical characteristics.

To conclude, until gene therapy is proven safe and effective through further basic research, it will remain an experimental treatment only used in clinical studies. Although there is still a long way to go before the RNAi approach will be employed in clinical conditions, scientists believe that it will eventually be used as a viable preventive or therapeutic intervention for serious diseases in the near future [1, 7, 11, 36].

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