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Liposomal Gene Delivery System

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P. Akshaya, K.K Anjima*, Nethaji Ramalingam, K.R Vimal

Devaki Amma Memorial College of Pharmacy, affiliated to Kerala University of Health Science and Approved by AICTE & PCI . Pulliparamba-673634, Malappuram Dt. India.

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ABSTRACT

Gene therapy is a therapeutic approach to deliver genetic material into cells to alter their function in the entire organism. The Gene delivery system is a unique way to use the adjustable gene to cure any disease. Many viral and nonviral vectors have been used to deliver genes to targeted sites. Liposomes are a novel drug delivery system which are vesicular structures consisting of hydrated bilayers are used for this. The success of liposome-mediated gene delivery is a multifactorial issue and well-designed liposomal systems might lead to optimized gene transfection particularly in vivo. Liposomal gene delivery systems face different barriers from their site of application to their target, which is inside the cell. However, in this review, we emphasize on the liposomal gene delivery system.

INTRODUCTION:

The changes in medicine, pharmacological treatment rapidly progresses into new fields. There is an emphasis on the development of treatment methods to eliminate underlying factors rather than to treat the symptoms of a disease. Therefore, research is increasingly utilizing knowledge from the field of genetics. Genetic mutation and deletion lead to many genetic disorders. Gene therapy is a therapeutic modality that relies on the successful delivery of nucleic acid agents to deliver genetic material into cells to alter their function in the entire organism. The main role of gene delivery research is to develop clinically relevant vectors that can be used to combat elusive diseases such as AIDS[1]. The development of an ideal carrier for effective delivery of therapeutic agents into diseased sites has always been a prime objective in any sort of therapy[2]. The carriers should selectively and efficiently deliver a gene to target cells with minimal toxicity to otherwise healthy normal tissue. Genetic materials are high molecular weight, polar compounds that do not permeate the biological barriers easily. Liposomes, the subject of the present review, are widely used in gene delivery[3]. This review has been concentrated on the development of multifunctional liposomes that target gene, a delivery system.

GENE THERAPY

The introduction of genes into cells of various origins has been a major technique in cell biology research for more than a decade. In addition to being a powerful research tool, gene transfer is a new concept for gene therapy, a molecular therapeutic approach for curing inherited, and many other diseases[4, 5]. A gene cannot enter a cell by itself for two reasons: it is a large piece of DNA and is surrounded by various anionic charges. Since gene therapy has progressed rapidly into clinical trials in a relatively short time, the methods of gene introduction must be compatible with therapy. This encouraged the development of nonviral DNA mediated gene transfer techniques such as liposomes.

Non-viral gene delivery systems were introduced as an alternative to viral-based systems. One of the most important advantages of these systems is improved transfection. Non-viral systems are categorized according to preparation, as physical or chemical types. The most common physical methods are microinjection, electroporation, ultrasound, gene gun, and hydrodynamic applications. In general terms, physical methods refer to the delivery of the gene via the application of physical force to increase the permeability of the cell membrane.

In contrast, chemical methods utilize natural or synthetic carriers to deliver genes into cells. In this method, polymers, liposomes, dendrimers, and cationic lipid systems are used as gene delivery systems. Gene delivery systems can be classified into two categories i.e., viral and nonviral vectors[6]. Nonviral vectors composed of cationic liposomes and polymers have many advantages, including being less immunogenic, biodegradable, and less toxic[7].

Gene delivery systems use various methods to allow uptake of the gene that has been selected to target the cell. The successful design of a gene delivery system requires a complete understanding of the interaction mechanism between the target cell and the delivery system. Understanding intercellular traffic and targeting mechanism is the most important factor in designing a more effective gene delivery system. Cell targeting refers to the delivery of the therapeutic agent to a specific compartment or organelle of the cell. It is the most commonly used mechanism in endocytosis gene therapy, particularly in cellular uptake of non-viral gene delivery systems.

In general, there are two non-viral vectors such as liposomes and polymers. Liposome based non-viral vectors use liposomes to facilitate gene delivery by the formation of lipoplexes. The lipoplexes form spontaneously when the negatively charged DNA contacts with positively charged liposomes. The polymer-based non-viral vectors use the polymers to interact with DNA to form polyplexes.

A successful gene therapy needs a necessary achievement to develop the proper gene delivery system. The gene delivery system is capable of cell specificity that can deliver an adequate amount of transgene expression to cause the desired effect [8]. The gene delivery system has utilized to generate a hybrid biosynthetic vector to deliver a possible vaccine as an application method. The vector overcomes the traditional barriers to gene deliveries by combining E. coli with synthetic polymers to create some vectors [9]. The gene therapy system gives a great opportunity for treating some diseases from a genetic disorder, cancer, and other infections.

The liposomal delivery has merit in its low immunogenicity and rather a low-cost reproducibility [10]. It has no limitation in DNA size for packing and modification with ligands to specific cell targeting[11]. The gene delivery systems based on synthetic polymeric materials have tested and evaluated for gene transfer to animals[12]. One of the candidates is the plasmid

DNA that can carry the gene into the nucleus of the desired cells safely. The gene delivery systems based on non-viral vectors have also risen as a new promising therapeutic modality[13].

LIPOSOME AS A CARRIER

Liposomes are colloidal drug delivery systems. They are biologic membrane-like sacs in sphere form, formed by one or more lipid layers, and include an aqueous phase. The phospholipid phase consists of principle components like aqueous phase and cholesterol. Liposomes are classified according to the number of layers they contain. The advantages of liposomes include effectiveness at small doses, extended dosing interval, and ideal transport for active substances with a short half-life. Cellular uptake mechanism of active substances in liposomes can be categorized as endocytosis, combination by melting, and adsorption. Liposomes are used for the application of carcinogenic, antifungal, antiparasitic, antiviral, and anti-inflammatory drugs, hormones, DNAs, and cosmetics[14]. Cationic lipids, which are used for the entrance of plasmid DNAs in the cell, have been studied for more than 20 years. During this period, many cationic liposome formulations were developed and were tested as nucleic acid transported in animals and humans with phase I and phase II studies. When compared to other gene delivery systems such as viral vectors and transfection agents, cationic liposome delivery systems are more easily formulated and cause no biological damage like viral vectors[15]. Critical parameters that determine the behavior of liposomes in in-vitro and in vivo conditions include size, number of layers, and surface charge. Unilamellar or multilamellar vesicles can be produced depending on the preparation method; the diameter of these vesicles ranges from 25 nm to 50 μm[14]. Liposomes are divided into three categories according to their charges: cationic, anionic, or neutral. Compared to viral vectors, liposome delivery systems are non-pathogenic and nonimmunogenic, with ease of preparation. However, the most important disadvantage of liposomes is short gene expression time and low transgene expression level. Since liposomes are generally non-toxic and non-immunogenic structures, they are considered reliable carriers for gene therapy. Liposome-based gene delivery was used in numerous gene therapy tests.

LIPOSOMAL GENE DELIVERY

Liposomes are composed of one or more simple or functional concentric lipid bilayer membranes that sandwich hydrophilic spaces in amongst them. Solubility and method of the formulation will define that drugs can be incorporated in either the aqueous or the

hydrophobic phase[16,17]. Morphology variation and size difference of liposomes may vary based on the lipid composition of the liposomes, formation condition of vesicles, the proportion of lipids to genetic material, intrinsic molecular weight and structure, and size of the genetic payload[18,19,20]. Liposomes might be applied in other formulations such as gels for transdermal drug delivery or bioerodible hydrogels for controlled release of nanoparticles to increase their durability in the application site, plasma, or other organs[21,22]. Such formulations also affect the properties and fate of liposomes.

From gene transfer to gene therapy:

A gene cannot enter a cell by itself for two reasons: it is a large piece of DNA and is surrounded by various anionic charges. For in vitro gene transfer, a variety of artificial techniques such as direct DNA microinjection, membrane perturbation by chemicals (organic solvents, detergents), physical means (mechanical or osmotic means, electric shocks) and liposomes have been used. However, in vivo, the interactions between DNA and cells may be more complex [23] have reported that naked DNA injected into the muscle of animals was expressed as protein. Despite the electrical repulsion between cell surfaces and DNA, it appeared that a few cells were able to assimilate the molecule. This suggests that a small amount of tissue damage or increased pressure at the injection site could play a role. In vivo interactions of DNA with proteins could also modify the interactions between cells and DNA. So, theoretically, it should be possible to inject naked DNA intramuscularly but the production of high local protein would be insufficient to be effective after its dilution in the bloodstream. Therefore, to improve gene delivery, DNA compaction with polycations, DNA encapsulation into recombinant retroviruses [24], adenoviruses, or liposomes have been evaluated. As far as retroviruses and adenoviruses are concerned, the possible production of recombination and the oncogenic effects of random insertion into the host genome have limited their use[25]. Since gene therapy has progressed rapidly into clinical trials in a relatively short time, the methods of gene introduction must be compatible with therapy. This encouraged the development of nonviral DNA mediated gene transfer techniques such as liposomes.

The successful gene transfer in vitro involves:

1) the packaging of DNA, 2) the adhesion of packaged DNA to the cell surface, 3) internalization of DNA, 4) escape of DNA from endosomes if endocytosis is involved, 5)

DNA expression in cell nuclei. To perform all of the above steps, liposomes have been explored as a delivery system for DNA[26]. The encapsulation of plasmid DNA into liposomes and the introduction of poliovirus RNA and DNA into cells via liposomes[27, 28].

Liposomal spherical nucleic acids (LSNAs), LSNA has the advantage of biocompatibility and at the same time has the general properties of the latter[29] and is therefore often used in immunotherapy. LSNA is more potent than linear nucleic acid in activating immune cells like macrophages[30]. The synthesis method of LSNA is generally to anchor a nucleic acid modified with a hydrophobic component such as cholesterol to a lipid bilayer of a liposome template. Nevertheless, the mobility of the liposome nucleus and the hydrophilic nucleic acid shell make the structure inherently less stable, limiting the widespread applications of LSNA[31]. The problem of stability become one of the barrier for the use of LSNA.

BARRIERS FOR GENE DELIVERY

Several barriers need to be overcome to increase the effectiveness of non-viral vectors in humans. Anatomic barriers are extracellular matrixes coating the cells, which prevent direct transport of macromolecules to target cells through the epithelium and endothelial cell sequences. Phagocytes like Kupffer cells in the liver and residential macrophages in the spleen are responsible for the clearance of DNA-loaded colloidal particles in blood circulation. Similarly, nucleases found in blood and extracellular matrix cause free and unprotected nucleic acids to be rapidly inactivated following systemic application. The most critical barrier to effective DNA transfection was regarded as the transition of the plasma membrane. Typically, naked nucleic acids cannot cross the cell membrane by cellular uptake mechanisms such as endocytosis, pinocytosis, and phagocytosis without the application of physical methods or being loaded to a carrier. An ideal gene delivery system should meet 3 criteria: The carriers should protect the transgene from nuclease enzymes inside intracellular matrixes; should transport the transgene from the plasma membrane to the target cell nucleus; and should not cause any toxic effect[33].

Systemic Barriers: After systemic administration of liposomes containing the genetic material, liposomes should stay intact in the blood, have little or no interaction with serum proteins, erythrocytes, and other cellular components and be able to reach the target tissue[34]. Genetic material has a short half-life in blood circulation because of rapid degradation by

nucleases[35, 36]. Substantial chemical modification of antisense molecules has overcome this obstacle.

Cationic liposomes can partially or fully protect associated oligonucleotides from degradation by serum nucleases and via selective delivery to target sites[37]. It has been shown that the intravenous application of liposomes have a more significant effect than naked DNA as these carriers prevent degradation of genes and promote cellular uptake[38,39]. In the case of the more commonly used cationic liposome-mediated nucleic acid delivery, the positive charge of the resulting complex (lipoplex), besides its benefits, also enhances non-specific electrostatic interactions of liposomes with serum components and molecules which result in a decrease of subsequent transgene expression in vivo[40]. The most important obstacle for liposomal nucleic acid delivery is the serum, a complex fluid containing lipoproteins, enzymes such as lipases and nucleases that can degrade liposomes and the genetic payload and therefore interfere with transfection efficiency[41-43].

Liposomes with neutral or anionic surfaces show enhanced stability in serum and increased circulation time but low loading of genetic material and reduced uptake by target cells, making them inferior to cationic liposomes. Other surface components and properties of liposomes are also important. In vitro experiments have shown that the glycol-coated liposomes are efficiently taken up by cells expressing carbohydrate-binding receptors selectively. Biodegradable agents such as polyhydroxy ethyl L-asparagine/L-glutamine and polyethylene glycol (PEG) attached by a hydrolyzable bond such as an ester to the liposome surface have the advantage of prolonged circulation in serum and diminish binding to the cell surface or deleterious opsonization, and binding and internalization to target tissue and cells[44,45].

Incorporation of PEG into the liposome diverts its accumulation in the lung to distal solid tumors[46]. It has been shown that for in vivo gene delivery via upstream intra-arterially administration, tumor uptake can be enhanced by docking of liposomes on to microspheres[47]. To reach the interstitial spaces of tumors, liposomes must pass the 50–100 nm thick glycocalyx shield on the luminal side of endothelial vessel first[48]. Relatively high interstitial fluid pressures and the organization of the interstitial environment are hurdles that keep liposomes from accumulating in target cells at high concentrations. One other obstacle in the blood is clearance from the blood by the kidney and reticuloendothelial system (RES: lungs, liver, and spleen) and extravasation in organs other than those constituting the RES.

Attachment of some hydrophilic components such as PEGs to the liposome can reduce uptake by RES. Even after upstream intra-arterial administration for genetic drugs, limited targeting and selectivity for cancer have been achieved[49].

Manipulation of liposomal structure and composition has been known to promote specific delivery or targeting. Without targeting moiety, deposition in capillary beds of the lung, and subsequent release into the plasma and clearance by spleen and liver due to its size and a high charge is likely a big obstacle in many cases[50]. Targeting ligands can be added either by directly coupling the ligand to the phospholipids or distal end of the PEG-lipid; more accessible for interaction with the receptors.

Cellular barriers: When liposomes reach a target cell, it has to overcome certain barriers for successful transfection. These barriers include (a) binding of the liposome to the cell surface, (b) entry of the liposome into the cells by endocytosis or direct traversing of the plasma membrane (e.g. via membrane fusion), (c) escape of the liposome from the endosome, (d) dissociation of the liposome to release nucleic acid payload, (e) transport through the cytosol and (f) entry into the nucleus.

CONCLUSION:

Gene therapy is a therapeutic approach to deliver genetic material into cells to alter their function in the entire organism. The development of an ideal carrier for effective delivery of therapeutic agents into diseased sites has always been a prime objective in any sort of therapy. One promising form of the gene delivery system is liposomes. Liposomes are promising nanocarriers in gene therapy. The success of these systems depends on the liposome properties, administration route, and the barriers that they face to reach their target inside the cells. For successful liposomal gene delivery, the barriers should be well understood. Compared to viral vectors, liposome delivery systems are non-pathogenic and non-immunogenic, with ease of preparation. Liposomes are generally non-toxic and non-immunogenic structures. Several barriers need to be overcome to increase the effectiveness of non-viral vectors in humans. Liposomes can protect associated from degradation by serum nucleases and selective delivery to target sites. Here we reviewed the liposomal consideration as an ideal vector for gene delivery.

CONFLICT OF INTEREST:

The authors confirm that this article content has no conflict of interest.

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