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Role of cationic lipids for the formulation of lipoplexes

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ABSTRACT

Cationic lipids are widely used for their advantages over viral gene transfer as they are non-immunogenic and their production is easy. The formation of cationic liposomes to lipoplexes with the help of cationic lipids has been done. Cationic lipids are often used in combination with helper lipids such as DOPE or cholesterol for defining their structural properties. The mode of lipoplex formation has been described in this review. This review also focuses on the parameters that affects the physico-chemical properties of lipoplexes describing their use for the cationic lipid based on the gene therapy purposes. It also focus on the mode of formation of lipoplexes with the help of various cationic lipids. The cationic lipids play a major role as compared to the anionic lipids. They play a vital role for the gene delivery. The current prospectus for the lipoplexes formation is also described. The non-immunogenic cationic lipids are more advantageous from others. The current status and various prospects for the transfection efficacy of lipoplexes is also been described.

Keywords: Cationic lipids; lipoplexes; non-immunogenic; transfection; liposomes.

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INTRODUCTION

For the improvement of the delivery of new DNA into the cell, the DNA must be protected from its damage and its entry into the cell must be done properly. The new molecules, lipoplexes, have been originated that have the ability to save the DNA from undesirable degradation during the transfection. Cationic lipids, because of their positive charge, form natural complex with the negatively charged DNA. Also as a result of their charge they interact with the cell membrane, endocytosis of the lipoplex occurs and the DNA is released into the cytoplasm. The cationic lipids also act as a saver against degradation of the DNA by the cell. Cationic lipids are the lipids which are amphiphilic in nature.[1] A huge amount of work has been done for the benefit of novel formulations of cationic liposomes, through the formation of various cationic lipids with less toxic substances and having various possibilities to mediate gene movement.[2]

Besides DOTMA (2,3-bis(oleoyl)oxipropyltrimethylammoniumchloride) andDOTAP(N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammoniummethyl-sulfate) are the two most famous cationic lipids, whose acyl chains are linked to the propyl ammonium group,various new lipids have become available for transfection purposes.[2] Cationic lipid or lipoplexes have been the subject of inquiry in recent years to understand the parameters governing the efficiency of transfection. Cationic lipids are sometimes used in combination with helper lipids such as DOPE(dioleoylphosphatidylethanolamine) or cholesterol. [3] Cationic lipid-mediated gene transfer is mainly used for their advantages over viral gene transfer because it is non-immunogenic, easy to produce and they are not oncogenic. The main drawback of the application of cationic lipids is their low transfection efficiency. [4] The main knowledge is of the structure-activity relationships of lipoplexes and of the mechanisms involved in the process of intracellular gene delivery is short. It is believed that such

type of knowledge is crucial to improve the biological activity of these systems and therefore gaining perception into these mechanical aspects should constitute one of the main goals in this field. Since 1987, when a cationic lipid was reported for the use to formulate the first vesicle used for introduction of plasmid DNA (pDNA) into cells.[5] Cationic liposomes can combine DNA into a cationic particle when the two components are mixed together. This cationic lipid/DNA complex (lipoplex) can save DNA from enzymatic degradation and deliver the DNA into cells by involving with the negatively charged cell membrane. Lipoplexes are not an ordered DNA phase that is been enclosed by a lipid bilayer and they are a partially concised DNA complex with an instructed substructure and with an asymmetrical morphology.[6]

All cationic molecule based systems have been disappointed or they have not been good to influence the clinical trials due to their low gene transfer efficacy and toxicity of substances associated with infection. Although the beginning of toxicity is not fully understood, a combination of unmethylated nucleic acids and the cationic molecules and maybe the larger diameter of the lipoplexes, play a major role in the inauguration of the toxicity.[7] Although the majority of clinical trials have been based on the use of viral vectors cationic liposomes are appearing as promising non-viral carriers for genetic medicines due to their safety and versatility.[8]

Lipoplexes likely enter cells mainly by adsorptive endocytosis.[9] The intracellular route is not yet clear-cut; but, it is fair that only a small fraction of the lipoplexes is able to avoid lysosomal degradation and to finish up in the nucleus.[10] The transfection capacity of lipoplexes in vitro depends on many framework, such as their physicochemical characteristics, type of cells, and incubation conditions.[11] However, of all the currently available relevant information, the logical way to boost lipofection *in vivo* is still not clear.

From cationic liposomes to lipoplexes

As many attempts had been made to use standard liposomes for gene transportation, the little skillfulness of plasmid–DNA condition and therefore the low plane of transfection pleased researcher to develop other liposome-based scheme.[12] As Behr[13] demonstrated that cationic liposomes, could complex and condense DNA, Felgner and collaborators proposed the use of cationic liposomes as economical transport for the intracellular transportation of DNA.[14] These authors prepared liposomes composed of the cationic lipid 2,3-bis(oleoyl)oxipropyl trimethylammonium chloride (DOTMA) and dioleoylphosphatidylethanolamine (DOPE), which became commercially available as a transfection reagent designated lipofectin. The ability of this system to mediate transfection was attributed to recognition of certain properties, namely: (1) a spontaneous electrostatic interaction between the positively charged liposomes and the negatively charged DNA, which results in an efficient condensation of the nucleic acids; (2) the fact that the resulting cationic liposome/DNA complexes could exhibit a net positive charge that promotes their association with the negatively charged cell surface and (3) the fusogenic properties exhibited by the cationic liposome formulation that can induce fusion and destabilization of the plasma membrane, thus facilitating the intracellular release of complexed DNA. These assumptions sealed the way to pursue studies aiming at improving the biological activity of lipoplexes, which allowed the identification of several critical parameters that affect their efficacy.

Liposome composition

Cationic lipids

Over the past some years a huge sum of work has been done for the improvement of novel formulations of cationic liposomes, that is to say through the analysis of various cationic lipids with low toxicity and showing various quality to mediate gene movement.[15] DOSPA and DOGS, which are ambiguous cationic lipids, signifies micellar instead of vesicular structures and display a high effectiveness in compressing DNA than univalent lipids. This place, however, does not surely evidence to a high transfection ratio, since the intracellular separation of DNA from the compound is suppose to be much more than challenging.[16] In general, the transfection activity of cationic lipids decreases with increasing alkyl chain length and saturation. Shorter alkyl chain length approves higher rates of intermembrane transfer of lipid monomers and lipid membrane mixing. A nonstop connection betwixt the universe of the tied unit of cationic lipids and their possible toxicity was also revealed. Lipids with constant ether attachment are more than poisonous than those including reactive ester attachment.

Co-lipids

The value of associating a co-lipid to finer the quality of cationic lipids to transfer cells has been demonstrated. *In vitro* survey clearly display that liposomes combined of an equimolar mixture of DOPE and cationic lipids can intermediate at high plane of transfection than those including only the cationic lipid or a different supporter lipid like DOPC.[17] This information has been assigned to the quality of DOPE to facilitate the formation of liposomes in conjuction with cationic lipids and to its inclination to go through a conversion from a bilayer to an hexangular conformation below acidic pH, which may serve merger with or modification of victim tissue layer, in particular endosomal tissue layer.[18]

More recently, it has been recommended that DOPE can also show a role in assisting the set up of the lipid based DNA formulations later their incorporation

and escapism of DNA from endocytotic cyst.[19] Cholesterol has also been hired as a co-lipid to prepare cationic liposomes, resulting in the formation of more lasting but less efficient complexes than those containing DOPE. In contrast, cholesterol-containing complexes have showed the high natural activity compared to complexes with DOPE when these complexes were used *in vivo*.[20]

Structure and size of cationic liposomes

The way by which the structure and size of cationic liposomes to transfect cationic liposomes affect the physico-chemical properties and biological activity of the lipoplexes is an important issue that needs to be clarified. In the great bulk of transfection studies lipoplexes are processed from large unilamellar cationic liposomes (LUVs), possessing sizes close to 100 nm, and from small unilamellar cationic liposomes (SUVs) with sizes ranging between 20 and 100 nm. It has been demonstrated that for a given liposome mixture, multilamellar liposomes (MLVs), which usually exhibit an average size ranging from 400 to 800 nm, when complexed with DNA intermediate high transfection activity than compound prepared from SUVs.[21] However, a novel method to modify the properties of lipid–DNA complexes was recently proposed, consisting of both bulge of the liposomes (to obtain large unilamellar liposomes, LUVs) and contained mixing of lipid and DNA. This procedure has been shown to result in lipoplexes showing small sizes within a constricting distribution that present a high colloidal stability and equal to features for their *in vivo* use.[22] Recently, it was rumored that lipoplexes resulting from MLVs do not disagree importantly in their magnitude or in the level of their cell organisation and consumption. Similarly, the transfection activity mediated by the resultant complexes was observed to be dependent on the final size of the complexes and not on the type of liposomes used.[23]

Although recent models proposed for the of the lipoplexes, which identify them as multilamellar aggregates, were settled on studies using SUVs, recent findings declared that in the process of lipoplex formation, DNA induces the generation of multilamellar liposomes from unilamellar vesicles.[24] Thus, the outcome of using MLVs instead of SUVs on the final properties of the complexes remains to be clarified.[25] The apparent discrepancy of results reinforces the need of further research focused on the understanding and control of pharmaceutical variables involved in the preparation of the cationic lipid-based gene delivery systems.[26]

Mode of lipoplex formation

It is well recognized that the mode of formation of complexes strongly determines the final physicochemical features of the lipoplexes and, consequently, modulates their biological activity.[27] In the last few years a significant effort has been devoted to gain insights into the parameters that affect the formation and the resulting structure and morphology of the lipoplexes.[28] Both experimental and theoretical studies have been performed and different models have been proposed for the cationic lipid–DNA complexes.[29]

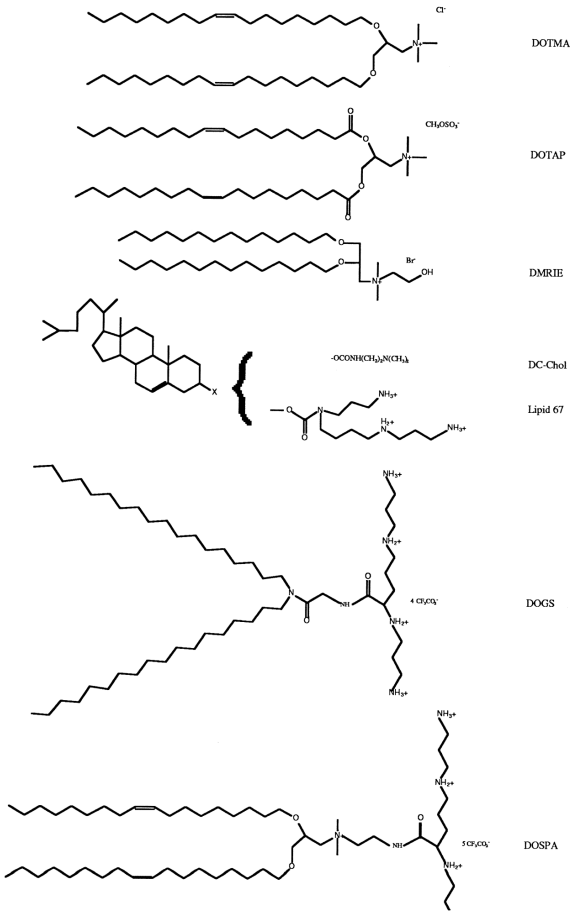

Figure 1: Composition of some cationic lipids usually used in gene therapy

Initially, based on light scattering results that indicated a slight size increase of the lipoplexes compared to the cationic liposomes, the calculation of the number of positive charges per liposome, as well as the possibility of complete charge neutralization for lipoplex formation, it was proposed that the lipoplexes resulted from the constricting of four entire cationic liposomes to the plasmid–DNA strand mediated by electrostatic interactions.[30] Gershon and collaborators,[31] by using electron microscopy, proposed a different model for the formation of the complexes according to which, at low lipid /DNA (+/-) charge ratios, cationic liposomes are adsorbed at the surface of DNA molecules forming aggregates that progressively surround large segments of DNA. The continuous addition of liposomes to a critical concentration and density results in DNA-induced membrane fusion and liposome mediated DNA collapse and condensation. According to these authors, these processes result in coating of DNA with a cationic lipid bilayer along the full length of the plasmid.

Similar observations were also made by Sternberg and collaborators who, using freeze fracture electron microscopy, described the morphology of the lipoplexes as aggregates of cationic liposomes encompassing DNA molecules (designated 'bead on string' arrangements) that coexist with tubular structures composed of DNA molecules coated by lipid bilayers. This gave rise to the so-called 'spaghetti-meatballs' model.[32] More recently, an important breakthrough towards understanding the interactions between DNA and cationic liposomes as well as the structure of the resulting lipid /DNA complexes was provided by a combined *in situ* optical microscopy and X-ray diffraction approach. The mixture of cationic liposomes with DNA results in a topological transition from the liposomal structure to a liquid-crystalline, concentrate and round construction, position of DNA mono layers, defined by a consistent inter-helical spacing, which are prepared betwixt cationic lipid bilayers. This structure was designated the $L\acute{a}^{c}$ structure.[33]

More recently, the role of DOPE in the lipoplex formation was far investigated by performing circular dichroism measurements. These studies showed that the involvement of DOPE in cationic liposomes causes, in addition to secondary conformational changes observed for pure DOTAP liposomes, a tertiary DNA transition that results from the embedding of DNA within the lipid columnar inverted -hexagonal assembly, which in turn provides the DNA with a given spatial organization and fixed directionality.[34] The authors proposed that this DNA packaging mode is interconnected and co-exists with short unbound segments of DNA which appear along the hexagonal assembly and meet into a highly condensed bundle characterized by a left handed chirality.[35] Overall, these findings contribute to rationalize the choice of the 'helper' lipid in the cationic liposomes.

Another interesting contribution to the understanding of the mode of lipoplex formation was reported.[33,34] Based on experimental evidence provided by cryo-electron microscopy and light scattering analysis, these authors described a novel morphology for cationic liposome–DNA complexes consisting of condensed DNA in the interior of illuminated liposomes between two lipid bilayers. As described above, these authors also found that the final structure of the lipoplexes is dependent on the liposome composition. Despite the various morphologies for the lipoplexes presented in the literature and summarized above, it is not yet possible to accurately define which are the conditions or factors that find out each of them. In fact, it is reasonable not to take out the possibility that the different structures co-exist in the same preparation and that the observed differences can be attributed to the lack of control of the different variables involved in the complex preparation, as well as to the fact that different techniques have been utilized to study lipid bilayers and thus to evaluate lipoplex morphology.[36]

Evidence in support of this hypothesis has recently been reported by Fang and Yang who have directly representated DNA on lipid membranes by atomic force microscopy (AFM), showing clear-cut ordered domains.

Parameters affecting the physico-chemical properties of lipoplexes

The physico-chemical characteristics including size, charge density and colloidal stability are relevant properties of lipoplexes that determine their successful use both *in vitro* and *in vivo.* Therefore, understanding the parameters that modulate such properties is of crucial importance.

Like in other systems, the physico-chemical properties of the lipoplexes depend on several thermodynamic as well as kinetic factors that affect complex formation, which therefore should be considered in their preparation. In this context, although several questions still remain to be clarified, it is well known that the concentrations of cationic lipid and DNA, the ionic strength and temperature of the suspending medium, the order of addition, the mixing rate of the components, and the extent of complex formation represent critical parameters.[37]

The relative proportion of cationic lipid and DNA determines the properties of the lipoplexes, namely size, surface charge (zeta potential), efficacy of complexation, colloidal stability and biological activity. Recent studies have shown that highly positively charged complexes, in which DNA is completely sequestered and condensed, exhibit an homogeneous size distribution (mean diameter between 100 and 450 nm). A similar size distribution is also observed when complexes are prepared with an excess of DNA over cationic lipids (i.e. negatively charged complexes), although in this case the presence of free DNA is generally observed.[38] On the other hand, complexes prepared from a lipid /DNA charge ratio of approximately 1/1 exhibit a neutral zeta potential, suggesting that all the cationic lipid molecules are neutralized by DNA. Such neutral complexes are characterized by a heterogenous size distribution (mean diameter from 350 to 1200 nm) and usually present a much lower colloidal stability than those exhibiting an excess of net positive or negative charge. This can be attributed to a lack of electrostatic repulsive forces among the complexes that would prevent their aggregation.[39]

Table 1: Marketed Products

Sno.	Clinical products	Approval year	Administration	Active agent	Company	References
1.	Doxil®	1995	I.V.	Doxorubicin	Sequus Pharmaceuticals	[44]
2.	DaunoXome®	1996	I.V.	Doxorubicin	NeXstar Pharmaceuticals	[45]
3.	Depocyt®	1999	Spinal	Cytarabine/Ara-c	Sky Pharma Inc.	[46]
4.	Myocet®	2000	I.V.	Doxorubicin	Elan Pharmaceuticals	[45]
5.	Mepact®	2004	I.V	Mifamurtide	Takeda Pharmaceuticals Limited	[47]
6.	Marqibo®	2012	I.V	Vincristine	Talon Therapeutics Inc.	[47]
7.	Onivyde™	2015	I.V	Irinotecan	Merrimack Pharmaceuticals Inc.	[48]
8.	Abelcet®	1995	I.V	Amphotericin B	Sigma Tau Pharmaceuticals	[44]
9.	Ambisome®	1997	I.V	Amphotericin B	Astellas Pharma	[46]
10.	Amphotec®	1996	I.V	Amphotericin B	Ben Venue Laboratories Inc.	[46]

The influence of lipid /DNA stoichiometry on the physico-chemical properties of the complexes becomes even more difficult to evaluate considering that, for a fixed lipid /DNA charge ratio, the increase in concentration of lipid and DNA results in a significant change of their size and colloidal stability, which can be attributed to enhanced precipitation at higher concentrations due to smaller interparticle separation.[40] Since thermodynamic parameters have been recognized to be involved in the formation of lipoplexes, the DLVO theory has been applied to understand how different parameters affect the final properties of lipoplexes.[41] In this regard, lipoplexes are usually prepared in low ionic strength solutions in order to decrease precipitation. Curiously, the precipitation effect caused by high ionic strength is particularly more pronounced during complex formation than in the stability of pre-formed lipoplexes, despite the the fact that under the same conditions they still obey the DLVO theory.

At low ionic strength, the attractive electrostatic forces required for lipoplex formation are enhanced, thus leading to a faster and more intense interaction between DNA and cationic liposomes, which seems to prevent the aggregation and sedimentation of the complexes. Temperature has been described not to be a critical parameter affecting lipid–DNA complex formation as well as their final properties, as long as DNA denaturation is avoided.

Regarding the effect of kinetic parameters, it is well established that rapid mixing of components results in small lipoplexes, while a very slow mixing often results in precipitation.[42] By analogy to crystal growth, this can be explained by the slow growth of a few nucleation embryos and critical ratios on a local scale that favour aggregation. In an attempt to prevent the formation of neutral lipoplexes that tend to aggregate, empirical rules have been followed regarding the order of addition of the lipid and DNA. If positively charged complexes are desired, DNA should be added to a cationic liposome suspension. The inverse will be true to obtain negatively charged complexes.

Production of liposomes for gene delivery

Based on the content with liposomal drug delivery systems, it is visualized that the ideal liposomes for systemic gene delivery will enclose plasmid DNA with high efficiencies, will protect the DNA from degradation by plasma nucleases, will have a thin size distribution, averaging 100 nm or less in diameter, in order that the liposomes can access extravascular regions, and will have the potency to incorporate a wide range of lipids, that promote fusion with cellular membranes and/or enhance liposome constancy in the circulation.

The feasibleness of passively encapsulating DNA in liposomes was demonstrated in the late 1970s using a number of the methods indicated. For example, high molecular weight DNA is trapped in egg phosphatidylcholine liposomes by hydrating the lipid film in the presence of DNA.[43] Reversed-phase evaporation procedures have also been employed to enclose plasmid DNAs with good but variable encapsulation efficiencies.

More recently, freeze drying methods have proceeded DNA-containing multilamellar vesicles with

encapsulation efficiencies of 50–60%.[44] For the most part, however, these operations yield relatively large multilamellar vesicles with low DNA encapsulating efficiencies and generally low gene transfer capabilities. Extrusion of the DNA containing multilamellar vesicles to cut down the particle size have resulted in poor recoveries of DNA containing liposomes.

In the late 1980s, it was shown that cationic lipids, when merged in dioleoylphosphatidyl- ethanolamine (DOPE)-containing liposomes, could raise the efficiency of gene delivery to cultured cells *in vitro* by (i) increasing the organization of plasmid DNA with liposomes and (ii) increasing the constricting of cationic liposome–plasmid DNA complexes to cells. This has prompted many researchers to analyze different cationic lipids that possess improved gene transfer and cell tolerability properties, as well as to develop novel procedures to efficiently encapsulate plasmid DNA drug inside lipid-based carriers.

Some properties of lipoplexes

They are non-immunogenic. They protect genetic material from being degraded as they are cationic they easily form complexes with negatively charged genetic material. The disruption of the endosomal membrane encouraging the endosomal escape is done by the lipoplexes. They communicate with cell membrane that clear the way for the endocytosis.[49] They are difficult to produce as compared to other complexes. They are more toxic as compared to other lipids. They become inactive in the presence of serum. They have lower transfection efficiency compared with viral vectors.

Applications

- Lipoplexes are usually prepared in low ionic strength solutions in order to decrease precipitation.
- The most common use of lipoplexes has been in gene delivery into cancer cells, where the supplied genes have activated tumor suppressor control genes in cell and decrease the activity of oncogenes.
- Recent studies shows that lipoplexes are useful in transfecting respiratory epithelial cells, so they may be used for treatment of genetic respiratory diseases such as cystic fibrosis.
- Most of the applications are based on the various types of gene delivery of the lipoplexes and their compounds that are been used for the drug delivery.
- They are mainly based on the type of various compounds used for the delivery of genes.[49]

Production of lipoplexes for conventional drug delivery

The major progression in lipoplex technology in the past has originate from the ability to produce well defined liposomes composed of a broad variety of lipids with various physical and chemical properties. Another significant advancement has come from the ability to entrap drugs in liposomes with high efficiency. Hydrophobic drugs can be directly incorporated into liposomes during vesicle formation. Trapping efficiencies of all drugs are often achievable, but are dependent on the solubility of the drug in the liposome membrane.[47,48]

CONCLUSION

Although over the last few years it has been difficult to demonstrate the synthesis of lipoplexes with the help of cationic lipids. This review describes the various cationic lipids that are used for the gene delivery. It focuses on the various aspects with the effect of liposome composition and with helper and co-lipids. It also describes structure and size of cationic liposomes with the mode of lipoplex formation. It also briefly describes about the various parameters that affects the physico-chemical properties of lipoplexes. As stated above,to achieve such a goal, attempts have been made to present viral attributes to lipoplexes.

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