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Review

Toxicity of cationic lipids and cationic polymers in gene delivery

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Abstract

Gene therapy, as a promising therapeutics to treat genetic or acquired diseases, has achieved exciting development in the past two decades. Appropriate gene vectors can be crucial for gene transfer. Cationic lipids and polymers, the most important non-viral vectors, have many advantages over viral ones as non-immunogenic, easy to produce and not oncogenic. They hold the promise to replace viral vectors to be used in clinic. However, the toxicity is still an obstacle to the application of non-viral vectors to gene therapy. For overcoming the problem, many new cationic compounds have been developed. This article provides a review with respect to toxicity of cationic lipids and polymers in gene delivery. We evaluate the structural features of cationic compounds and summarize the relationship of toxicity and structure and hope to provide available suggestions on the development of these cationic compounds.

Keywords: Gene therapy; Non-viral vector; Cationic lipids; Cationic polymers; Toxicity

Contents

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1. Introduction

Gene therapy has become a promising strategy for the treatment of many inheritable or acquired diseases that are currently considered incurable. The main objective in gene therapy is successful in vivo transfer of the genetic materials to the targeted tissues [1–4]. However, naked therapeutic genes are rapidly degraded by nucleases and show poor cellular uptake, so that the development of safe and efficient gene carriers is one of the prerequisites for the success of gene therapy [3–6].

Biological carriers are viruses, which are naturally evolved to infect cells and transfer their genetic materials into the host cells. Both RNA and DNA viruses have been evaluated as possible gene carriers. They are, however, difficult to produce and toxic (in particular immunogenic), as well as having a limitation in terms of the size of the inserted genetic materials [1,2,7]. In attempts to overcome these problems, non-viral vectors, such as cationic lipids and polymers, have been developed as gene carrier molecules. Non-viral vectors are advantageous due to the low immune response that enables repeated administration and the capability of large production with acceptable costs [1,2,8]. They have the potential to be widely used in clinic of gene therapy.

However, the present study is mainly focused on the experiments in vitro; toxicity is still an obstacle to the application of non-viral vectors to gene therapy [8–13]. Toxicity, the capacity of a drug to damage or cause adverse effects in the body [14], is a dose-relative notion. We often evaluate toxicity with lethal dose, threshold dose and maximal no-effect dose. Toxicity effect can be classified into acute effect and delayed effect, and also can be local effect and systemic effect. Local toxicity refers to the adverse effect on the site of injection, while systemic toxicity refers to damage in other organs when the poison is distributed through circulation system in body. Cationic lipids and cationic polymers for gene delivery may cause toxic effect in vitro and in vivo. For example, lipoplexes caused several changes to cells, which included cell shrinking, reduced number of mitoses and vacuolization of the cytoplasm [15]. Certain proteins such as protein kinase C may also be affected detrimentally by cationic amphiphiles [16].

In the following sections, we will discuss the toxicity of main cationic gene vectors (cationic lipids and cationic polymers), emphasis is placed on the relationship between toxicity and structure of these compounds. We evaluate the structural features of cationic compounds and discuss which groups may increase the toxicity, what kind of linkages have relatively short half-life, and how proper modifications will decrease the toxicity of cationic lipids and cationic polymers for gene delivery.

2. Cationic lipids

In 1987, Felgner et al. [17] first reported the utilization of unnatural diether-linked cationic lipid, *N*-[1-(2,3-dioleyloxy) propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA), as a synthetic carrier to deliver gene into cells. Since then, a series of cationic lipids have been synthesized for gene delivery. In comparison with other gene delivery modes, such as viral vectors, cationic lipids are simple and quick to formulate, are not as biologically hazardous as viral vectors, are readily available commercially, and may be relatively easily adapted for specific applications [1,2,8].

2.1. Lipid structure vs. toxicity

Cationic lipids used for gene therapy are composed of three basic domains: a positive charged headgroup, a hydrophobic chain, and a linker which joins the polar and non-polar regions [18]. Fig. 1 displays the three basic domains of 1,2-dioleoyloxy-3-trimethylammonium propane (DOTAP). The polar and hydrophobic domains of cationic lipids may have dramatic effects on both transfection and toxicity levels.

2.1.1. Hydrophobic chain

There are two major types of hydrophobic moieties, namely aliphatic chains and cholesterol-based derivatives. Traditionally, for aliphatic chains, single-tailed cationic lipids are more toxic and less efficient than their double-tailed counterparts. Pinnaduwage et al. [19] reported that cetyl trimethylammonium bromide (CTAB) was more toxic and less efficient than DOTMA. However, Tang and Hughes [20] demonstrated that 6-lauroxyhexyl ornithinate (LHON) with one tail was more efficient and of lower cytotoxicity compared with DOTAP (Fig. 1). This result shows that we cannot completely abolish the possibility of one tail cationic lipids for gene therapy application (Fig. 2).

Some of lipids, such as derivatives of cholesterol, are protein kinase C (PKC) inhibitors, which may be associated with their toxicity. Cationic amphiphiles containing steroid backbones were more potent inhibitors of PKC than their straight-chain analogues, therefore they had higher toxicity too [21]. Three novel galactosylated cholesterol derivatives, cholesten-5-yloxy-*N*-(4-((1-imino-c-β-D-thiogalactosyl-ethyl)amino)butyl)formamide (Gal-C4-Chol) and its ethyl formamide and hexyl formamide analogues (Gal-C2-Chol, Gal-C6-Chol) were synthesized by Kawakami et al. Liposome/DNA complexes prepared with these lipids showed low cytotoxicity in human hepatoma Hep G2 cells [22].

The effect of hydrophobic chain on toxicity has not been adequately addressed to date. Many scientists have been trying to give a proper explanation; however, there is still a long way to go. In any case, the influence of hydrophobic chain length on

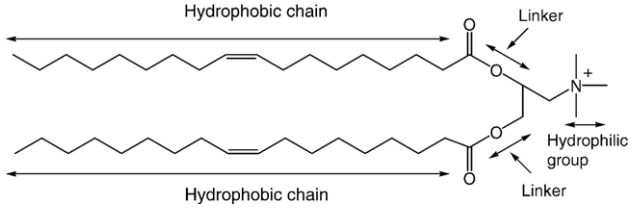

Fig. 1. Schematic representation of DOTAP, a commonly used cationic lipid for gene delivery.

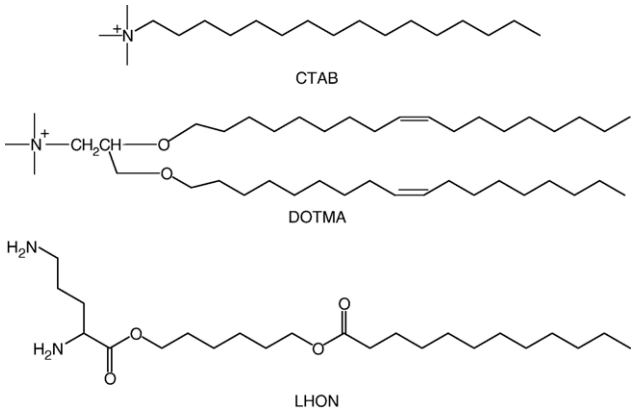

Fig. 2. Chemical structures of CTAB, DOTMA and LHON.

the parameter may well depend on the physicochemical features of the other two domains [23].

2.1.2. Hydrophilic group

The cytotoxic effect is associated with the cationic nature of the vectors, which is mainly determined by the structure of its hydrophilic group. The headgroup often consists of primary, secondary, tertiary amines or quaternary ammonium salts, but guanidino and imidazole groups have also been trialed [24]. In addition, Floch et al. [23] developed a class of cationic lipids characterized by a cationic charge carried by a phosphorus or arsenic atom instead of a nitrogen atom.

The cationic lipids can become cytotoxic by interacting with critical enzymes such as PKC. The research shows that many derivatives of cholesterol which contain tertiary or quaternary nitrogen headgroups can inhibit PKC activity. Quaternary ammonium amphiphiles are more toxic than their tertiary amine counterparts [21]. A recent solution to circumvent these problems was to spread the positive charge of the cationic head by delocalizing it into a heterocyclic ring. Heterocyclic cationic lipids containing imidazolium or pyridinium polar heads [25–28] have been reported to display higher transfection efficiency and reduced cytotoxicity when compared with classical transfection systems [29].

Ilies et al. [30] reported that 1-(2,3-dioleoyloxypropyl)-2,4,6-trimethylpyridinium lipid (2Oc) (Fig. 3), a kind of pyridinium lipid, was able to transfect several cancer cell lines with similar or better efficiency than DOTAP, while producing lower cytotoxicity.

In addition, guanidine and its salts are important intermediates for organic synthesis and medicine; they are also used as cationic headgroups for making cationic lipids. Yingyongnarongkul et al. [31] synthesized three libraries of guanidinium-containing transfection agents, among which they found the library with two headgroups and one tail were most effective for transfecting mammalian cell lines. Compared with detergents which produce a high degree of toxicity, this variety of compounds were safer to use in vivo.

The toxicity of cationic lipids is mainly determined by their cationic nature. The quaternary amine headgroup is more toxic than tertiary one. The import of a heterocyclic ring as the substitution of the liner amine headgroup, such as pyridinium and guanidine, can spread the positive charge of the cationic head, and then toxicity is decreased significantly.

2.1.3. Linker bonds

Most of the linker bonds in the above mentioned synthesized lipids are ether, ester or amide bond. Although compounds with ether linker render better transfection efficiency, they are too stable to be biodegraded thus cause toxicity. Cationic lipids with ester bonds such as DOTAP in the linker zone are more biodegradable and associated with less cytotoxicity in cultured cells [32–35], but those with ester or amide linkers are liable to decompose in the circulation system.

In recent years, carbamate-linked lipids which with lower toxicity [36–38] (Fig. 4) as novel cationic lipids have been developed. It is familiar to chemists that compounds comprising carbamate bond is stable in the neutral circumstance and is liable to acid-catalyzed hydrolysis. As well known, the pH value in endosomes is 1–2 lower than that of the circulation system [39,40], and it is expected that these carbamate-linked lipids can keep stable in the circulation system while decompose to release DNA after entering endosomes in cell because of the pH decreasing [36]. The lipids may be rapidly degraded into nontoxic low molecules in cell.

Aberle et al. [16] proposed that cytotoxicity due to cationic lipids may occur at a stage before the lipoplexes were encapsulated into endosomes. An increase in the length of the linker segment led to decreased toxicity in cell culture [23]. These results show that the cytotoxicity is lowered while the linkage is degradable.

2.2. Liposomes vs. cytotoxicty

Cationic lipids could be used in either the form of liposomes, or DNA/lipid complexes formed by the interaction of positively charged lipids at the physiological pH with the negatively charged DNA through electrostatic attractions. The transfection efficiency with these delivery vehicles in vitro is, in part, determined by their stability and particle size. However, these

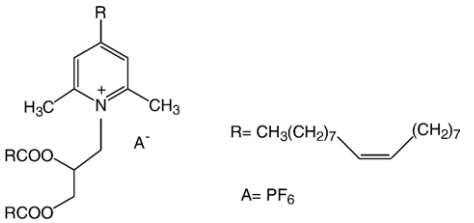

Fig. 3. 2Oc—a kind of pyridinium lipid.

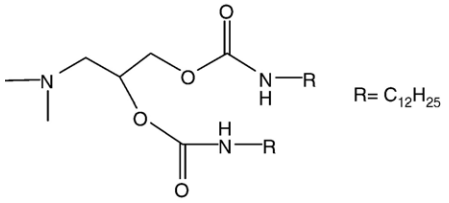

Fig. 4. A kind of carbamate-linked lipid.

liposomes or DNA/lipid complexes often exhibit reduced transfection efficiency in vivo, and also cellular toxicity [42]. For example, serum proteins can decrease transfection efficiency by neutralization of the positive zeta potential [41], and by binding to and increasing the particle size of the complexes. The toxicity may, in part, result from the large size of the complexes, and the high positive zeta potential required for their uptake [43]. The toxicity is normally closely associated with the charge ratio between the cationic lipid species and the nucleic acids, as well as the dose of lipoplexes administered [44]. Higher charge ratios are generally more toxic to a variety of cell types. In addition, different reagents have different degrees of toxicity to cells, and toxicity is cell-specific.

2.2.1. Free liposomes

Research revealed that the toxicity is associated with free liposomes. Removal of free liposomes from DOTAP/DNA lipoplexes prepared at high positive/negative ratios did not improve the transfection efficiency compared with plain lipoplexes prepared at lower ratios [45]. It allowed, however, the use of lipoplexes with high positive/negative ratios was presumably due to a reduced toxicity of the purified lipoplexes, while avoiding the decrease in transfection efficiency that was observed for plain lipoplexes [45]. This result suggests that the toxicity associated with lipoplexes prepared at high positive/negative ratio is essentially associated with free liposomes. Another possibility is that when free liposomes are present, they may compete with lipoplexes for binding/uptake by the cells.

2.2.2. Co-lipid in synergy with cationic lipids

Neutral lipids are often a component for cationic liposome formulations in which they play an assistant role. Three neutral lipids often incorporated into formulations are dioleoylphosphatidylethanolamine (DOPE), dioleoylphosphatidylcholine (DOPC) and cholesterol (Fig. 5).

Mukherjee et al. [46] experimented on electrophoresis gel patterns in DNase I sensitivity assay and proved that the high transfection properties of the present cationic lipids in association with the equimolar amounts of DOPE, cholesterol and DOPC may partly originate due to reducing DNase I susceptibility of the corresponding lipoplexes. The role of DOPE is to facilitate membrane fusion and aid the destabilization of the plasmalemma or endosome [47,48], while DOPC does not destabilize lipid bilayers [49]. DOPE-containing liposomes, as well as various galactosylated cholesterol derivatives, exhibited low toxicity and high transfection efficiency with regard to human hepatoma cells, Hep G2 [22,50]. Additionally, oligopeptides were also used as co-lipids in the research of Tokunaga et al. [51], which revealed that the ternary complex (DNA/oligopeptide/liposome) had high transfection efficiency and low cytotoxicity, higher protection from DNase I digestion and the less binding with serum proteins.

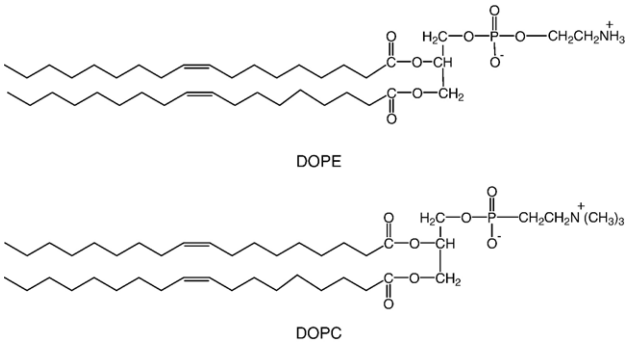

Fig. 5. Two commonly used neutral lipids, DOPE and DOPC.

The usage of neutral lipids allows one to decrease toxicity and attain higher transfection levels in vivo [52,53], which may be determined by their special structures. For instance, DOPE can facilitate membrane fusion and aid the destabilization of the plasmalemma or endosome. In a word, the use of co-lipids may turn out to be rewarding in design of novel liposomal transfection kits for gene delivery.

3. Cationic polymers

Cationic polymer (at physiological pH) can be combined with DNA to form a particulate complex, polyplex, capable of gene transfer into the targeted cells [2]. The most obvious difference between cationic polymers and cationic lipids is that they do not contain a hydrophobic moiety and are completely soluble in water [54]. Compared with cationic liposomes, they have the obvious advantage of compressing DNA molecules to a relatively small size [55,56]. This can be crucial for gene transfer, as small particle size may be favorable for improving transfection efficiency. Modifications to these polymers such as molecular weight, geometry (linear vs. branched) and ligand attachment can be easily achieved [18,54]. This opens the way to extensive structure/function relationship studies. The most widely studied polymers for gene therapy include poly (ethylenimine) (PEI) and poly(L-lysine) (PLL).

3.1. PEI

Polyethylenimine (PEI), a commercially available cationic polyamine first introduced by Boussif et al. [57], is one of the most successful and widely studied gene delivery polymers [58–60]. There are mainly two types of structure: Linear molecule and branched molecule. PEI is a gene carrier with high transfection efficiency and high cytotoxicity [61]. Many factors affect the efficiency/cytotoxicity profile of PEI polyplexes such as molecular weight, degree of branching, ionic strength of the solution, zeta potential and particle size [59,62]. One study, for instance, showed that low molecular weight (10 kDa), moderately branched polymer resulted in efficient delivery with low toxicity in comparison with commercial high molecular weight PEI [63,64].

Godbey et al. [65] reported that there were mainly two types of cytotoxicities in process of PEI-mediated cell transfection. One was an immediate toxicity associated with free PEI, while the other was a delayed toxicity associated with cellular processing of PEI/DNA complexes [66]. When administered in the circulatory system, the free PEIs interacted with negatively charged serum proteins (such as albumin) and red

blood cells, precipitated in huge clusters and adhered to the cells surface [63]. This effect could destabilize the plasma-membrane and induced the immediate toxicity. Solutions proposed to overcome these problems include addition of PEG, and will be discussed later. But fortunately, when PEI was complexed with DNA, the immediate toxicity was decreased [66]. The delayed toxicity by PEI/DNA complex was closely related to the release of DNA from PEI [66]. Once out of the circulation, during internalization into the cell's cytoplasm and release of their DNA cargo, free PEI is restored. In cell culture, free PEI interacts with cellular components and inhibits normal cellular process. It causes several changes to cells, which include cell shrinking, reduced number of mitoses and vacuolization of the cytoplasm.

To solve these problems, Kim et al. [61] designed a class of degradable PEIs with acid-labile imine linkers (Fig. 6). The acid-labile PEI may be rapidly degraded into low molecular weight PEI in acidic endosomes. In toxicity assay, the acid-labile PEI was much less toxic than PEI 25 kDa, due to the degradation of acid-labile linkage. Therefore, the use of acid-labile PEIs may be hopeful for gene delivery.

The nature of PEI enables the researchers of successfully introducing targeting ligands and/or polyethylene glycol (PEG) to its surface, so that higher transfection efficiency and lower cytotoxicity are achieved. For instance, Kircheis et al. [62] linked PEGylated PEI polyplexes to tumor-specific ligand transferrin, an asialoglycoprotein and then applied intravenously, resulting in five-fold increase in the transfection efficiency with lower toxicity in comparison with PEGylated (transferrin-free) PEI polyplexes. The addition of PEG as co-polymer, which produces sterically stabilized gene carriers, can markedly decrease the toxicity.

As other cationic polymers, PEI is associated with dose-dependent toxicity, which probably explains why it has not yet been used in clinical studies. It has been reported that modifications of PEI, like PEG-grafted or biodegradable PEIs, led to a decrease in complex charges and much less cytotoxicity than bPEI after applying them at high concentration to mouse fibroblasts [67,68]. Some novel cationic polymers based on PEI showed higher transfection efficiency and low toxicity in vivo. Thomas et al. [69] compared branched PEI with deacylated PEI for gene delivery in mouse lung. They reported that polyplexes formed by branched PEI 25 kDa were highly toxic at N/P = 10, causing the death of all of the mice injected with them, while the fully deacylated PEI 25 kDa exhibited dramatically higher transfection efficiency and low toxicity. Tang et al. [70]

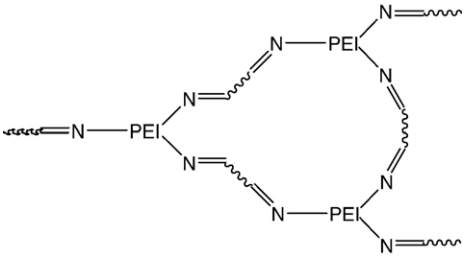

Fig. 6. Structure of acid-liable PEI.

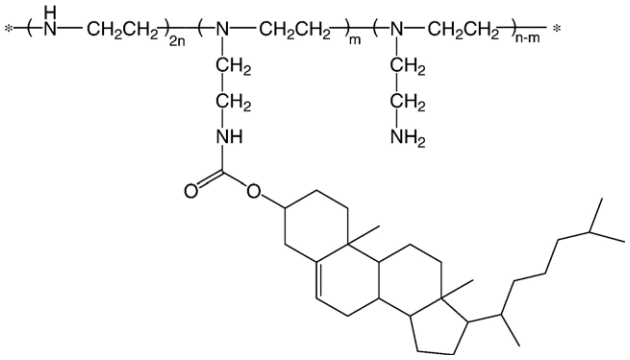

Fig. 7. Stucture of PEI-Chol.

developed and tested a new PEI polymer synthesized by linking low molecular weight PEIs with beta-cyclodextrin. The polymer displayed improved biocompatibility over non-degradable PEI 25 kDa and high transfection efficiency in cultured neurons and in the central nervous system of mice.

To combine the advantages of both cationic polymer and liposome, water soluble lipopolymer was designed [71,72]. Han et al. [71] synthesized a kind of PEI-Chol lipopolymer (Fig. 7) for gene delivery. PEI-Chol was a water soluble lipopolymer and non-toxic to a variety of cells [71–73]. PEI-Chol lipopolymer is amphiphilic in nature because PEI is hydrophilic and water soluble, while cholesterol is hydrophobic. With the increase in its concentration, PEI-Chol may form multimolecular micelles or micellar aggregates in water, which depended on the hydrophilic–hydrophobic balance between the cationic headgroup and the lipid tail. At high N/P ratios, some lipopolymers were present in the suspension of PEI-Chol/pNDA complexes in the free forms and could affect transfection and cytotoxicity [73].

Scientists are now working on the design of suitable novel water soluble lipopolymeric gene carriers by incorporating fusogenic peptide molecules [74], which may be more effective for gene delivery to human islets with little or no toxicity. In addition, adapting controlled release technologies to the delivery of DNA can reduce toxicity that limits gene therapy [75]. These systems typically deliver vectors locally, which can avoid distribution to distant tissues and decrease toxicity to nontarget cells. For example, in gene delivery system, adsorption of PEI/DNA complexes to silica nanoparticles [76,77] resulted in transgene expression in vitro comparable to that observed by bolus delivery and with reduced toxicity. The usage of these systems may promote the development of gene delivery.

3.2. PLL

PLL polymers are one of the first cationic polymers employed for gene transfer [78]. They are linear polypeptides with the amino acid lysine as the repeat unit; thus, they possess a biodegradable nature. This property is very useful for in vivo applications. However, when entered into the circulatory system, PLL polyplexes were rapidly bound to plasma proteins and cleared from the circulation [79]. This may cause lower

transfection efficiency. In addition, successful transfection still requires co-application of chloroquine, a lysosomotropic agent, which reduces the lysosomal degradation of lipoplexes [80,81]. This, however, causes an increase in toxicity [82].

Many PLL polymers with different molecular weights were tested and evaluated for gene transfer [83–85]. It has been shown that DNA condensation and transfection efficiency increased with high molecular weight PLL, which was also associated with undesirable high toxicity [86]. Okuda et al. [87,88] reported that dendritic poly(L-lysine) of the 6th generation (KG6) (Fig. 8) showed high transfection efficiency, without significant toxicity or cell specificity. Like amphiphilic PEI, the creation of amphiphilic PLL, by linking both PEG and palmitoyl groups to the polymer, reduced toxicity without compromising the gene delivery efficiency [89].

3.3. Cationic polysaccharides

Cationic polysaccharides, water-soluble and biodegradable cationic polymers, have been absorbed more and more scientists' eyes. The two typical classes are chitosan derivatives and cationic polymers based on dextran-spermine (D-SPM).

Chitosan, a naturally occurring linear aminopolysaccharide, has been shown to excel in transcellular transport [90,91]. In studying chitosan based controlled release systems, researchers found that apart from the biocompatibility, biodegradability and low toxicity, chitosan excelled in enhancing the transport of drugs across the cell membrane [92–94]. Most recently chitosan has been expanded to the field of gene transfection, and many encouraging results have been published. For example, Liu and Yao [95] reported that chitosan and trimethylated chitosan oligomers proved to be nontoxic on several types of cells in contrast to DOTAP that decreased viability to 50%.

Recently, scientists developed a novel water-soluble and biodegradable cationic polymer based on dextran-spermine (D-SPM) (Fig. 9) gene delivery. That novel polycation was capable of complexing and administering various genes to many cell lines in relatively high yields [96]. Yudovin-Farber et al. [97] synthesized cationic polysaccharides based on monoquaternary ammonium spermine, and found the compounds demonstrated moderate effect on the cell viability or no toxicity at all at 3:1 weight ratio (dextran-spermine/DNA). Further attempts to improve its performance by quaternorization of the spermine residue resulted in the loss of activity and increased toxicity.

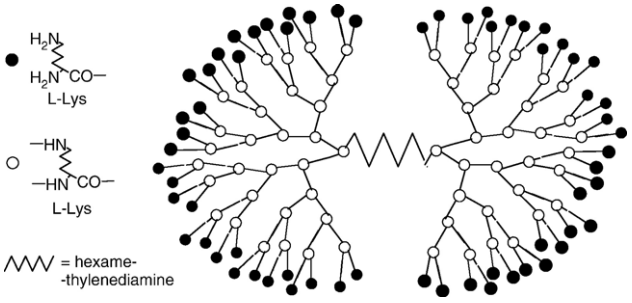

Fig. 8. Structure of dendritic poly(L-lysine) of the 6th generation (KG6).

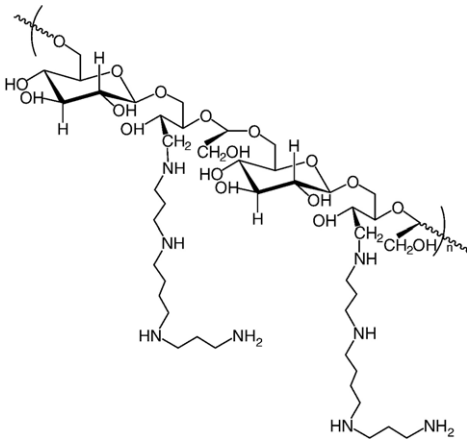

Fig. 9. Structure of D-SPM.

Compared with other cationic lipids or cationic polymers, D-SPM seemed to be lower toxic [98]. Eliyahu et al. [99] experimented on polyplexes based on D-SPM in mice, and evaluated local and systemic toxicity. Histopathological assays revealed mild toxicity in muscle, no abnormal findings in liver and lung, and no systemic toxicity obtained.

3.4. Other cationic polymers

Since the most important aspects of cationic polymers for gene therapy are transfection efficiency and toxicity, many new cationic polymers, such as acrylates and dendrimers (highly branched polyamidoamine) [100,101], have been developed aiming to improve transfection efficiency and to lower toxicity.

In recent years, scientists have developed polymethacrylates, such as pDMAEMA, pDAMA and the degradable pHPMA-DMAE, as gene delivery vectors. It was reported pDAMA and pHPMA-DMAE exhibited very low toxicity in vitro [102,103]. The study showed that pDMAEMA-based polyplexes possessed similar cytotoxicity and transfection activity as that of the frequently used transfectant PEI [104]. Funhoff et al. linked a membrane-disrupting peptide to different polymethacrylates to get a better endosomal escape and higher transfection efficiency. The toxicity of both the pDAEMA- and pDAMA-based polyplexes was not affected by the conjugation of the peptide. The pHPMA-DMAE-INF-based polyplexes showed substantial transfection activity and low toxicity at rather high polymer to plasmid ratios, this was also found for the unmodified polymers [82].

A novel polycation known as reducible polycations (RPC) prepared by oxidative polycondensation of the peptide Cys–Lys (10)–Cys resulted in enhanced transfection within various cancer cell lines in comparison with PLL [105]. It was believed that cellular reduction of disulfide bonds of these vectors could facilitate gene delivery and reduce cytotoxicity. Maheshwari et al. tested a nontoxic biodegradable polymer, poly[alpha-(4-aminobutyl)-L-glycolic acid] (PAGA), for murine interleukin-12 gene delivery to CT-26 tumor-bearing BALB/c mice [106,107]. Cytotoxicity was significantly reduced; however, transfection efficiency was not high enough to use this polymer in clinical therapy.

4. Discussion

Cationic lipids and cationic polymers are the most probable alternative to viral delivery systems and are increasingly being used in vitro and in vivo. However, in vivo nucleic acid delivery has been traditionally hindered by the toxicity associated with their formulations. In the past few years, modifications to commonly used delivery systems have been made and novel carrier systems have been developed to overcome this problem.

The toxic effect is mainly determined by the cationic nature of the vector, which attains different level to different structure. For cationic lipid, the cytotoxic effects are mainly determined by the structure of its hydrophilic group. Quaternary ammonium amphiphiles are more toxic than their tertiary amine counterparts. Importing a heterocyclic ring, such as imidazolium or pyridinium, can spread the positive charge of the headgroup, and then decrease the toxic level. The toxic effects are also determined by the compounds biodegradable ability. Carbamate-linked lipids may be rapidly degraded into nontoxic low molecules in cell and provide high transfection efficiency. The effect of hydrophobic chain on toxicity has not been adequately addressed to date. Like cationic lipid, cationic polymers with acid-labile linkage can be rapidly degraded and less toxic. Since the toxicity of PEI or PLL increases with high molecular weight, polymers synthesized by linking low molecular weight PEIs with acid-labile show low toxicity. The creation of amphiphilic cationic polymer based on PEI or PLL, by linking PEG or other groups, reduces toxicity without compromising the gene delivery efficiency. Cationic polysaccharides display high biodegradability and low toxicity, and may be widely used in the future. In these gene delivery systems, free liposomes(or free polymers) play an important role in toxic effect, so purifying them can significantly reduce toxicity. High transfection efficiency and low toxicity can be obtained by the addition of co-lipids or co-polymers, such as DOPE and PEG.

Cationic lipids with heterocyclic rings show low toxic level, and would be worth exploring further as an in vivo delivery system for gene therapy. The design of water soluble lipopolymer, to combine the advantages of both cationic polymer and liposome, is an embraving idea. The modification of some natural products may hold promising position for use in gene transfer. In accordance with this hypothesis, we are working on sucrose ester-derived compounds which have polyvalent cationic and anionic headgroups to coexist in these structures to lower toxicity and to maintain high transfection efficiency of these polycations. The headgroups are linked with the hydrophobic chain through carbamate bonds which provide the lipid with excellent biodegradability. These compounds will be tested for gene delivery in vitro and in vivo in the near future.

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