Expert Opinion

Peptide-based cationic liposome-mediated gene delivery

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Introduction: As an important type of nonviral gene delivery vector, peptide-based cationic liposomes have shown many advantages over other cationic liposomes, such as good biodegradability, excellent biocompatibility and targeting ability to cells, and great potential application in improving the delivery of gene therapeutics.

Areas covered: This article reviews the research progress on peptide-based cationic liposomes for gene delivery, including the structure characteristics of peptide lipids, the development of peptide cationic liposomes and three special types of peptide cationic liposomes: peptide-based Gemini lipids, lipitoids and lipids with cholesterol hydrophobic group. This review hopes to provide some suggestions on the design of peptide cationic lipids and insight into their development trend in the field of gene delivery.

Expert opinion: As peptide-based cationic liposomes still hold some limitations, future research needs to select suitable peptide heads and investigate the surface modification of peptide cationic liposomes, in order to facilitate targeting and reduce cytotoxicity.

Keywords: Gemini lipids, gene delivery, lipitoids, liposomes, peptide cationic lipids

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1. Introduction

Gene therapy requires safe and efficient carriers to transfer expressible genetic materials or silencing nucleic acids to target tissues. The most extensively used delivery tools can be generally divided into two categories, viral vectors and nonviral vectors. Viral vectors such as adenovirus vectors, adeno-associated virus vectors or retrovirus vectors have been utilized in clinical trials [1]. However, some side effects including immunoresponse and infection have been reported [2,3], and it is relatively difficult to get a large number of recombinant viral vectors. Nonviral gene delivery systems have, on one hand, been demonstrated as standard tools for *in vitro* transfection with currently more than 60 commercially available kits [4]. On the other hand, they are regarded as a promising approach for the treatment of genetic diseases [5,6] and cancers [7].

After several accidents [8-10], nonviral vectors gained great attention for they are nonimmunogenic, not oncogenic, easy to produce in large scale and capable to deliver large genetic materials. However, unlike viral analogs that have evolved to overcome cellular barriers and immune defense mechanisms, nonviral gene carriers consistently exhibit significantly lower transfection efficiency compared with viral ones. Therefore, people have been designing and synthesizing a large amount of new compounds including cationic lipids, which could be prepared into cationic lipsomes, which have been originally used for the drug delivery to provide long circulation time and targeting ability, and polymers used as nonviral vectors [11,12]; at the same time, the hybridized utilization of nonviral

Article highlights.

- Among nonviral vectors, peptide cationic liposomes have drawn great attention, as they show low cytotoxicity, high transfection efficiency and wide range of transfection.
- The future research still needs to select suitable peptide heads and to investigate the surface modification of peptides cationic liposomes for facilitating the targeting.
- Another trend is to continuously reduce the cytotoxicity through the design of head groups playing the key role of cytotoxicity.

This box summarizes key points contained in the article.

vectors may provide a solution to the problem. People could combine cationic lipids and helper lipids, hybridize cationic liposomes and polymers, and introduce peptides and targeting moieties into lipids for approaching the requirements of gene therapy [13].

Among these nonviral vectors, peptide cationic lipids have drawn great attention [14,15], as peptides often have a specific biological signal, and they can significantly improve the adhesion of cells on the surface. Peptides and their derivatives have been used for restraining cancer cell migration, curing antithrombosis, treating acute renal failure, reducing anti-inflammation and promoting skin regeneration, and so on. But the isolated small peptides are easily degraded by enzymes *in vivo*, and they will be cleared within few seconds [16]. People found that the introduction of peptide groups into cationic lipids can prolong their half-life *in vivo* and enhance targeting thereof. The value of cationic liposomes with peptides for the delivery of genetic materials has been realized gradually by researchers [17,18], as they have many advantages, including numerous free active functional groups on their surface, avirulence, use either *in vitro* or *in vivo* [19-21], no obvious limits for materials contained in size, no inflammation and the control of the amount of the materials into the cells [16]. Moreover, they maintain their physiological concentration advantages, most amino groups have been protonated in the process of carrying genes and thereby, with positive charge, they could combine with negatively charged plasmid DNA to form liposomes/DNA complexes by electrostatic attraction [19].

We have searched the literatures published and found no reviews elucidating specifically peptide-based cationic liposomes for the delivery of genes. We believe that the summary of peptide cationic liposomes could provide useful information for researchers and further improve the design of new compounds. In this paper, we state the utilization of peptide cationic liposomes for the delivery of genes from the chemical point of view; especially, we focus on three kinds of peptide cationic lipids: peptides-based Gemini lipids [22], lipids with peptoid head group (lipitoids) [23] and peptide lipids with cholesterol hydrophobic group [24,25].

2. Chemical structure of peptide-based cationic lipids

The structure of peptide cationic lipids generally comprises a hydrophilic cationic head group attached via a linker to a large hydrophobic domain (**Figure 1**). The polarity of head group plays the combination role between the liposomes and DNA, liposomes–DNA complex and cell membrane or other components within the cell. It showed the gene expression efficiency concerned with the amino acids or peptide cationic head group of lipids [26]. The commonly used head groups are amino acids, such as alanine, glycine, lysine, arginine, histidine, ornithine and tryptophan (**Figure 2**) or peptides consisting of these amino acids (**Figure 3**). These amino acids and peptide head groups are able to deliver oligonucleotides into cells by utilizing short sequences of basic amino acid residues, which readily cross the plasma membrane. In a study, to mimic structural characteristics present in nucleic acid binding protein, a tri-peptide, Lys-Trp-Lys, was used as the head group for the amphiphile because it possessed cationic charges and an aromatic side chain and was relatively small in size. Cytotoxicity experiments performed with all the tri-peptide lipophilic peptides showed no significant cytotoxicity, similar to nontreated cells and significantly better than Lipofectamine 2000 when exposed to Chinese hamster ovarian (CHO) or NIH 3T3 cells [27]. The linker determines the chemical stability of cationic lipids and biodegradation [28]. For all lipids used in gene transfection, the cationic head groups and hydrophobic portions are joined by several common linkers including ethers, esters, amides and carbamates [29]. For example, *N*-[1-(2,3-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride and dimethyl dioctadecylammonium bromide containing ether bonds or C–N bonds have favorably chemical stability, but cannot be biodegraded and, therefore, not suitable for *in vivo* use. Cationic lipids containing esters allowing for cleavage once inside the cell are more easily biodegraded, and they have little cytotoxicity, but their chemical stability is usually poor. Amide and carbamoyl bonds are often used as linkers, because they are chemically stable and biodegradable [30]. Cleavable linkers are of interest for designing complexes, which can easily release DNA after endocytosis. Such cleavable linkers include photo- and pH-sensitive, redox-reactive and enzymatically degradable groups [18]. In general, the hydrophobic domain in peptide-based cationic lipids is composed of two long aliphatic chains. Double-chain alkyls, generally 12 – 18 carbon units in length, can be either completely saturated or contain double bonds (e.g., oleyl group) and are not necessarily symmetrical [29]. Many studies have shown that the length and type of aliphatic chain affect the transfection efficiency of a given lipid. Since the work pioneered by Huang *et al.* [31], cholesterol and other steroids have been used in place of aliphatic chains to compose hydrophobic part [32,33].

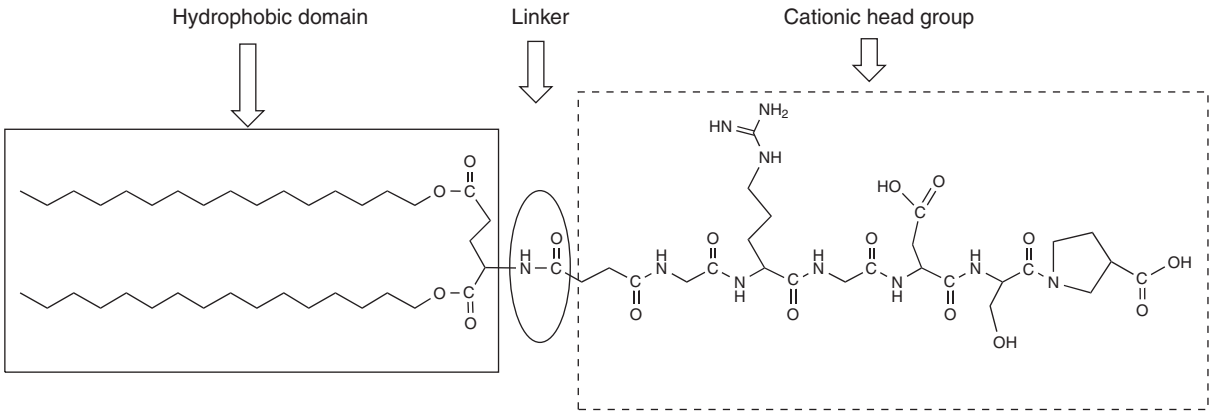

Figure 1. Schematic illustration of peptide cationic lipid (C16)2-Glu-C2-GRGDSP.

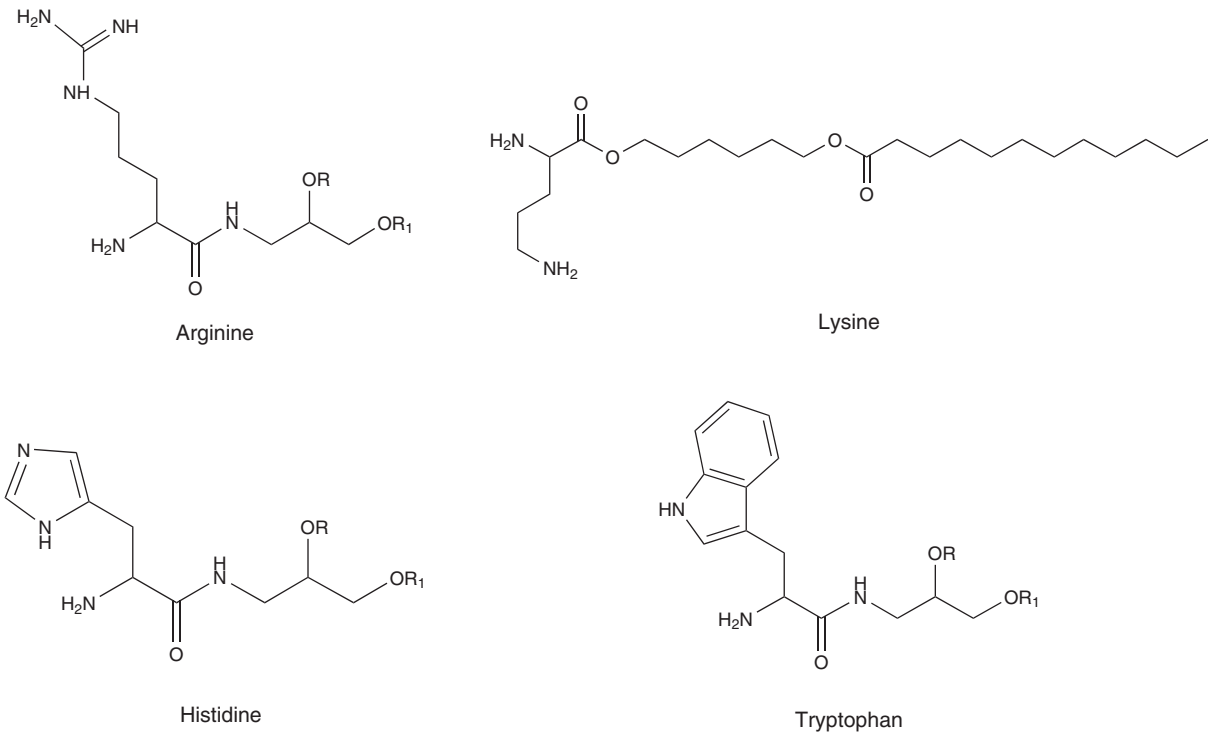

Figure 2. Lipids with typical amino acids head groups.

3. Progress of peptide-based cationic liposomes

Liposome-mediated gene transfer was one of the earliest strategies used to introduce exogenous genetic materials into host cells. Cationic liposomes are mostly utilized as gene carriers for plasmid DNA (pDNA) [34,35], antisense oligonucleotides [36] or small interfering RNA (siRNA) [37,38]. By 1987, the term 'lipofection' had been coined to describe lipid-based gene transfection [39,40]. Early work suggested that lipoplexes were delivered into the cytoplasm by direct plasma membrane fusion [41], but it is now agreed upon that liposomes-mediated gene transfer proceeds primarily through endocytosis [42,43]. Following cellular uptake, lipoplexes destabilize the endosomal membrane, resulting in a flip-flop reorganization of phospholipids. These phospholipids then diffuse into the lipoplex and interact with the cationic lipids causing the DNA to dissociate into the cytoplasm [44,45]. The mechanism of gene transfer of cationic lipoplexes has been thoroughly reviewed [46,47]. However, much of the work investigating

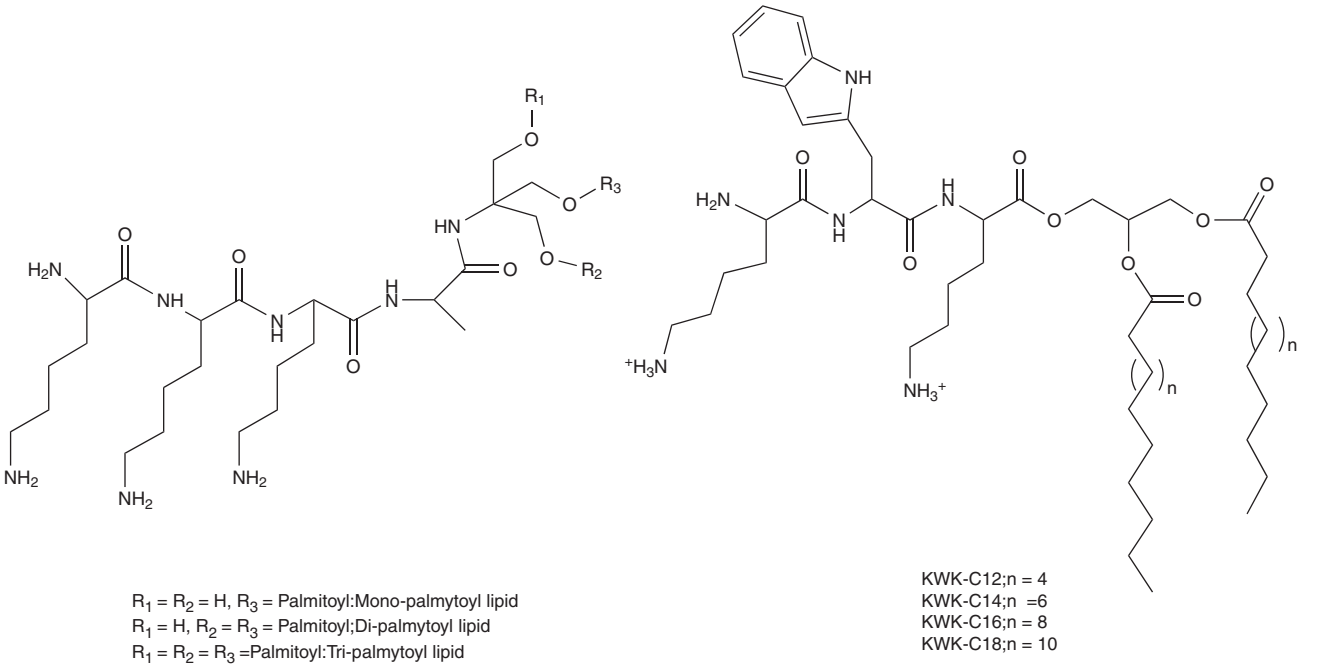

Figure 3. Lipids with typical peptide head groups.

the mechanism of nonviral gene delivery has focused on the intracellular barriers that a delivery vector must overcome to deliver its cargo to the nucleus, including binding to the cell surface and internalization [48], escape from the endosomal pathway [49-51], transport in the cytoplasm [52,53], nuclear entry [54] and vector unpacking [55].

The first polypeptide cationic liposomes prepared through a polycondensation reaction were described by Folda *et al.* [56]. They reported that the aminolysis of amino acid esters caused amino acid polycondensation on the surface of liposomes to give peptides modified liposomes. One of the problems with these vesicles was the fact that the amide head groups were not hydrophilic enough to avoid precipitation. Afterward, Neumann *et al.* [57] prepared amino acid liposomes by ultrasonication of amphiphilic amino acids with one additional hydrophilic unit at the polar head group. The conversion to peptide liposomes was achieved by using water-soluble carbodiimides as condensing agents. From then on, peptide liposomes have been found to have many advantages, such as good biodegradability, excellent biocompatibility and targeting ability to cells. Hence, peptide-based liposomes in gene delivery have been paid much more attention, as many new compounds have been designed and synthesized.

Kim *et al.* [58] synthesized a cationic lipid containing an aspartic acid backbone and a lysine head group; it can efficiently deliver genes and reduce the cytotoxicity. Later, they proved that the lipid with glutamate backbone was a more capable delivery system than aspartic acid. The lipid with glutamate backbone and its lipoplexes possessed greater bilayer integrity and stronger cationic surface charge than other lipoplexes. These characteristics appeared to be related to their strong binding affinities for cell surfaces, which then rendered them more effective in *in vitro* and *in vivo* gene transfection [59]. Rea *et al.* [14] reported on *in vitro* efficacies of nine novel non-glycerol-based cationic amphiphiles with increasing hydrophobic tails and the amino acids of serine, α-alanine and β-alanine as the head group functionalities in transfecting multiple cultured cells including CHO, COS-1, MCF-7 and HepG2. The structure–activity investigation demonstrated that high gene delivery efficacies of cationic amphiphiles containing α-alanine or β-alanine head groups can get seriously compromised by substituting α-alanine or β-alanine with serine presumably due to the enhanced sensitivity of DNA associated with such serine-head-containing cationic lipids.

Conjugation to cationic cell penetrating peptides (such as Tat, Penetratin or oligo arginines) efficiently improves the cellular uptake of large hydrophilic molecules such as oligonucleotides and peptide nucleic acids (PNAs), but the cellular uptake is predominantly via an unproductive endosomal pathway and, therefore, mechanisms that promote endosomal escape (or avoid the endosomal route) are required for improving bioavailability [60,61]. A variety of auxiliary agents (chloroquine [62,63], calcium ions [62] or lipophilic photosensitizers [64,65]) have this effect, but improved, unaided delivery would be highly advantageous in particular for future *in vivo* applications. To eliminate the utilization of these auxiliary agents, they conjugated a lipid domain (fatty acid) to the cationic peptide (a CatLip conjugate), which increased the biological effect of the corresponding PNA conjugates in a luciferase cellular antisense assay up to two orders of magnitude [66]. The effect

increased with increasing the length of the fatty acid (C_8 – C_{16}) but in parallel also resulted in increased cellular toxicity, with decanoic acid being optimal. To mimic structural characteristics present in nucleic acid-binding proteins, Prata *et al.* [27] have prepared a series of new lipophilic peptide vectors that possessed lysine and tryptophan amino acids for evaluation. These lipophilic peptides showed minimal cytotoxicity and enhanced *in vitro* gene transfection activity. Recently, Coles *et al.* [15] have synthesized positively charged peptide-based carriers including lipoamino acids (**Figure 4**). The carriers were shown to interact with DNA by performing isothermal titration calorimetry and particle size and zeta potential experiments. The particle sizes of the carrier/DNA complexes varied over the different charge ratios from 200 – 800 nm. The utilization of lipophilic carriers is a promising approach to improve the bioavailability of gene delivery.

Yosuke [67] synthesized cationic lipids bearing lysine, histidine or arginine as the cationic head groups for use in gene transfer studies. These lysine- or arginine-type lipids exhibited higher gene expression efficiency than that of Lipofectamine 2000, a conventional transgenic reagent. The gene expression efficiency in relation to the cationic head groups of the lipids was as follows: lysine > arginine > histidine. Moreover, the synthetic cationic lipids revealed remarkably low cytotoxicity compared with Lipofectamine 2000. Then, they synthesized a series of cationic amino acid-based lipids having a spacer between the cationic head groups and hydrophobic moieties and examined the influence of the spacer on a liposome gene delivery system. As a comparable spacer, a hydrophobic spacer with a hydrocarbon chain composed of 0, 3, 5, 7 or 11 carbons and a hydrophilic spacer with an oxyethylene chain (10 carbon and 3 oxygen molecules) were investigated. Plasmid DNA (QIAGEN Plasmid Mega kit)-encapsulating liposomes were prepared by mixing an ethanol solution of the lipids with an aqueous solution of pDNA. The zeta potentials and cellular uptake efficiency of the cationic liposomes containing each synthetic lipid were almost equivalent. The results proved that the cationic lipids with the hydrophobic spacer were subject to fuse with biomembrane-mimicking liposomes. 1,5-Dihexadecyl-*N*-lysyl-*N*-heptyl-L-glutamate, having a seven-carbon atom spacer, exhibited the highest fusogenic potential [67]. An oxyethylene chain spacer showed low gene expression efficiency and a hydrophobic spacer was a key component for improving pDNA delivery. More recently, they are investigating the ability of cationic liposomes composed of 1,5-dihexadecyl *N*-arginyl-L-glutamate (Arg-Glu2C_{16}) (**Figure 1**) to carry nucleic acids into neuronal cells. Lipoplexes between the Arg-Glu2C_{16} liposomes and plasmid DNA encoding green fluorescent protein (GFP) were analyzed in terms of lipoplex formation, intracellular DNA trafficking, transfection efficiency and cytotoxicity in neuronal SH-SY5Y cells. A maximum number of cells expressing GFP were obtained with lipoplexes at a lipid-to-DNA ratio of 15. With these lipoplexes, 16% of the cells were GFP positive, which was approximately fourfold higher than the level obtained with Lipofectamine 2000. Furthermore, as a result of the low cytotoxicity of the Arg-Glu2C_{16} lipoplexes, the proportion of GFP-positive cells could be increased to 25% by increasing the concentration of lipoplexes applied to the cells. Arg-Glu2C_{16}, as a model cationic amino acid-based lipid, had a high capability as a gene carrier, even for neuronal transfection [68].

4. Peptide-based Gemini lipids

Gemini lipids are a class of special cationic lipids in which two lipid molecules are joined to each other via a spacer. Though Gemini lipids have received significant industrial attention as emulsifiers and dispersants in detergents, cosmetics, personal hygiene products, coatings and paint formulations, recently they have been used as novel gene transfection agents due to their superior surface-active properties and DNA-binding capabilities [69]. Bhattacharya *et al.* [70] reported for the first time the synthesis and aggregation properties of various glycerol-backbone-based Gemini lipids. In their study, transmission electron microscopy (TEM) of their aqueous dispersions confirmed the vesicle formation, and from the thermal, spectroscopic, DLS and XRD studies it has been revealed that they formed three different kinds of membranous aggregate depending on the *m*-value.

People have synthesized some Gemini lipids among which peptide-based Gemini lipids have been paid much more attention especially those used in gene delivery. Kirby *et al.* synthesized five novel peptide-based cationic Gemini lipids and examined their ability to transfect plasmid DNA (pCMV) containing the luciferase gene. Three of these lipids, differing in the number of positive charges per molecule at neutral pH, mediated transfection on their own to show that their efficiency increased markedly on the addition of a neutral colipid and a basic polypeptide [71]. Then a study [72] on a class of Gemini lipids based on a peptide-like backbone has found that the optimal structure for transfection was with C18 hydrophobic chains and tri-lysine head groups linked via their side chain amine rather than normal peptide linkage (synthesis route is shown in **Figure 5**). Preliminary results suggest that combining Gemini lipids with dioleoylphosphatidylethanolamine (DOPE) should allow the preparation of liposomes of various sizes and lipid compositions. Control of such colloidal changes could be as significant as the changes in the molecular composition of the Gemini lipids in delivering optimum gene expression in animal models. Additionally, Buijnsters *et al.* [73] have synthesized cationic Gemini lipids with two cationic head groups and two alkyl chains connected by a tether (**Figure 6**) and described their aggregation in water, monolayer behavior, DNA binding and gene transfection activities. DNA binding was confirmed by the ability of all lipids used to release ethidium bromide from their complexes with DNA in an agarose gel electrophoresis experiment. The polylysine- and tartaric acid-based head groups have shown significant transfection efficiency.

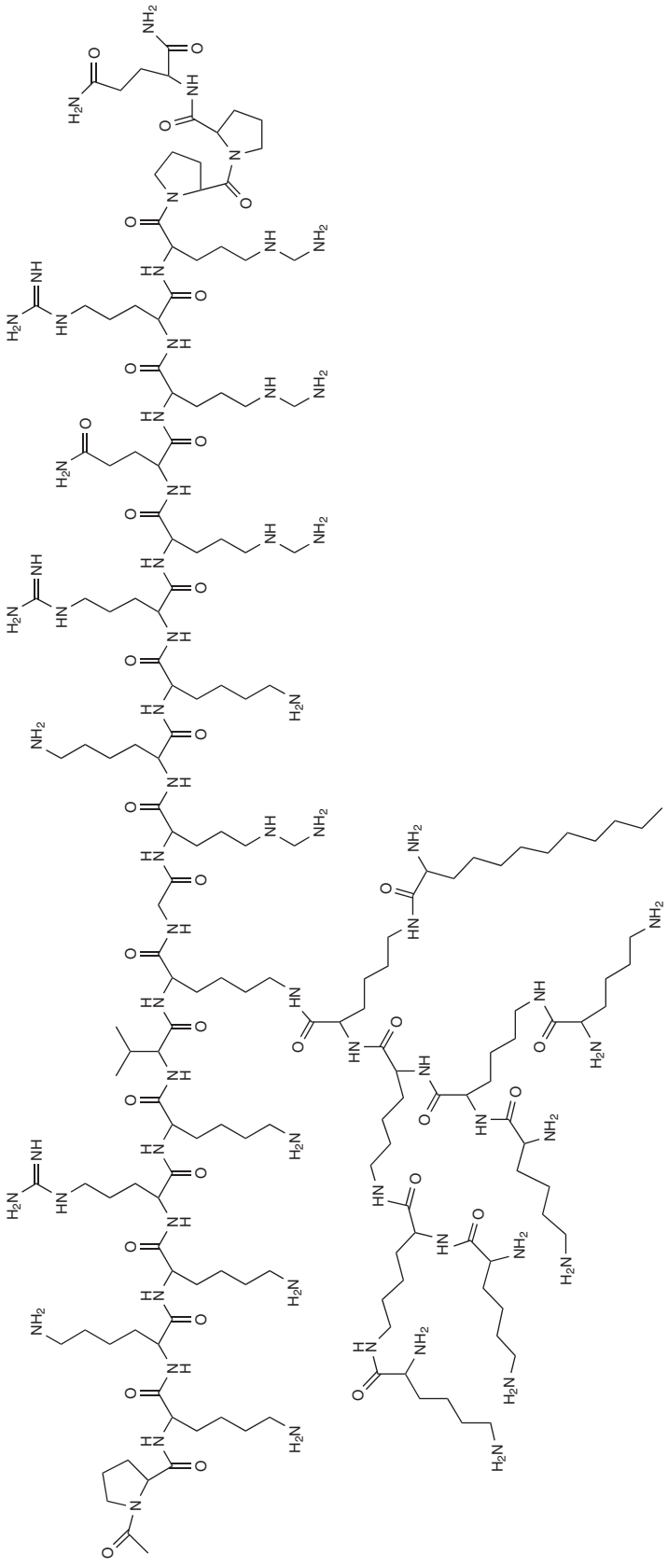

Figure 4. Complete structure of peptide-based carrier.

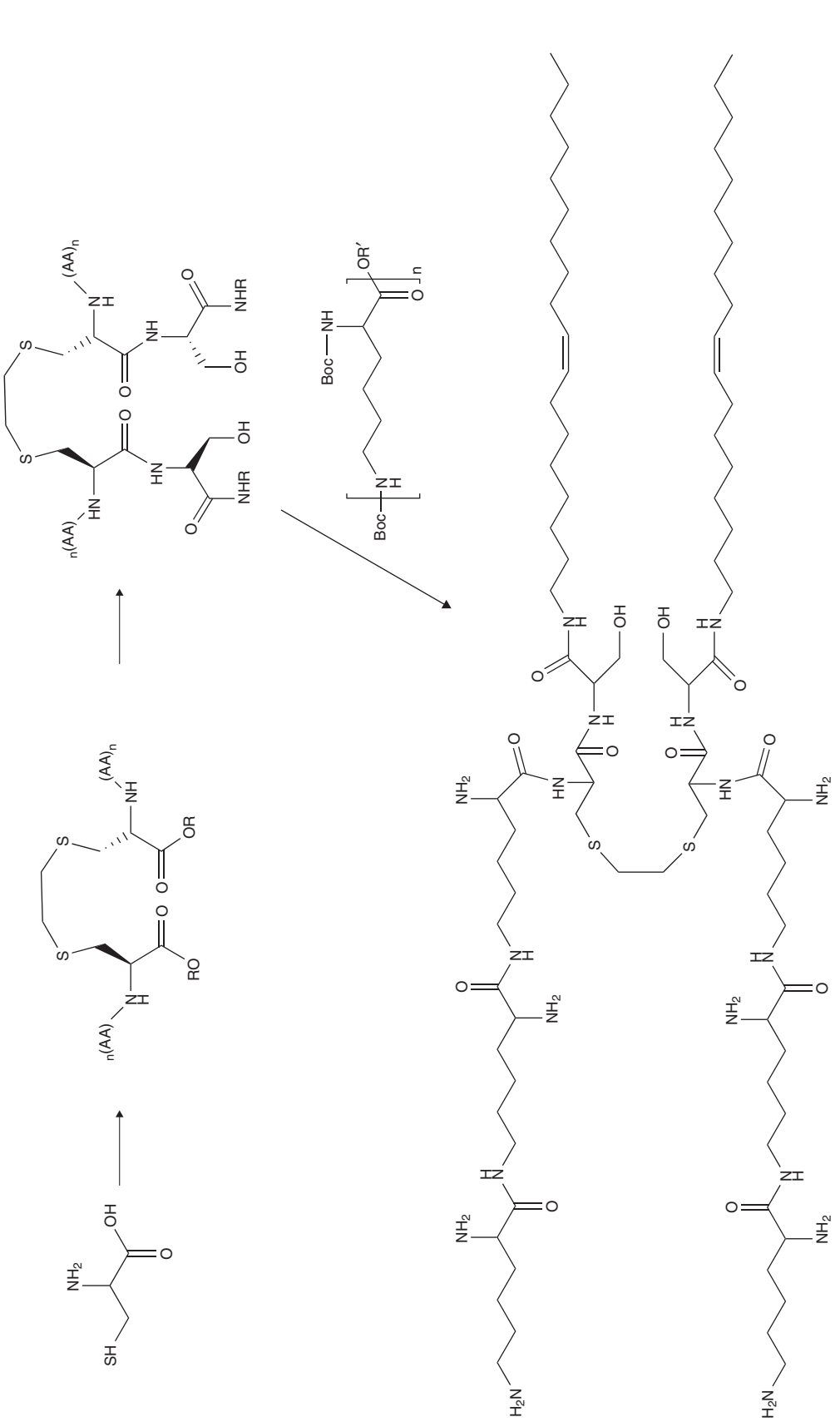

Figure 5. Scheme for synthesis of Gemini lipid GS11.

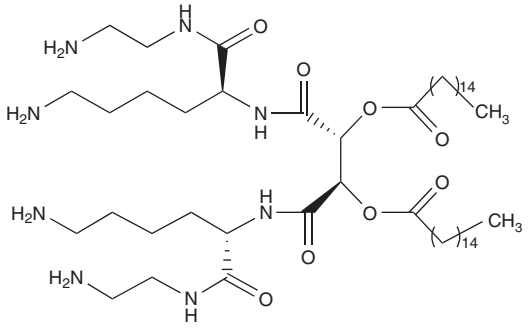

Figure 6. Structure of typical Gemini lipid.

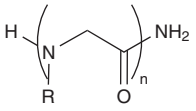

Figure 7. Chemical structure of peptoid N-substituted glycine oligomer.

Brito *et al.* reported that the aqueous self-assembly of a novel -lysine-derived lipid with a Gemini-like architecture, designated as 12-Lys-12, can be used alone or in a mixture with dodecyltrimethylammonium bromide (DTAB). For a mixing molar ratio in the range 2<DTAB/12-Lys-12<4, a region of stable, unilamellar vesicles can be found. Self-diffusion measurements and cryo-TEM imaging showed that the average vesicle radius was on the order of 30 – 40 nm, which may be beneficial to overcome the various barriers and the self-assembly with DTAB incorporated had high transfection efficiency for cells [74].

5. Lipitoids

Although synthetic nonviral vectors hold great promise for the delivery of nucleic acids, their gene transfer efficiencies are far from matching those of viruses. Therefore, researchers who are continuing to design and synthesize novel compounds among them lipitoids are gaining more and more interest. When peptoid head groups referred to as poly-*N*-substituted glycines (**Figure 7**) are connected to lipids, lipitoids are obtained. One of the chemical structures of lipitoids is shown in **Figure 8**. Their convenient synthesis enables strict control over the sequence of highly diverse monomers and is capable of generating extensive libraries of compounds (a typical synthesis route is shown in **Figure 9**). The potential ability to take advantage of specific peptide sequences to overcome extra- and intracellular barriers to gene delivery is also an advantage over other vectors.

Lipitoids were conveniently prepared in high yield using solid-phase synthesis by Huang *et al.* [23]. Several lipitoids condensed a luciferase reporter plasmid into 100-nm spherical particles and protected the DNA from DNase digestion. A peptoid motif formed by a trimer repeat of positive, neutral side chains was found to be the most efficient in DNA transfection assays, and higher charge density was detrimental to transfection [23].

It has been shown that peptoids exhibit unique pharmacokinetic properties, different from ordinary peptides, with greater protease resistance and membrane permeability being attributed to the lack of protons on the amide bond [75]. The N-substituted groups represent the secondary structural diversity of peptoids like peptide side chains, allowing for the construction of libraries of compounds and leading to the discovery of aptamers (or inhibitors) against therapeutic targets [76,77]. However, peptoids have intrinsic poor plasticity to form a variety of three-dimensional (3D) structures, and, therefore, the 3D structural diversity in the peptoid library would be limited [78]. Alternatively, a peptoid–peptide hybrid would be an attractive framework to improve the plasticity perhaps without sacrificing the major pharmacokinetics of the peptoid.

Lu *et al.* [79] made modification on the γ-N of the PNA backbone that yielded a PNA analog with a peptoid-like side chain. They found that the length of the side chain was important in influencing the hybridization affinity of the modified PNA. The amino head group in AP-PNAs (PNA containing at least one amino peptoid side chain) can be modified in various ways without affecting the binding affinity, suggesting that the peptoid side chain can bear a variety of functional moieties. Cationic peptoids were conjugated to phospholipids to give new lipitoids that were used as transfection agents for the delivery of RNA oligonucleotides in tissue culture experiments, permitting specific gene silencing. These 'lipitoid' reagents proved highly effective at mediating the specific knockdown of target genes. This effect was observed even in primary cell types that are highly resistant to the action of typical chemical transfection agents [80].

To systematically investigate the structure–activity relationship of cationic lipids, a small library of cationic lipitoids conjugates were synthesized. The compounds were evaluated for their ability to form complexes with plasmid DNA and to mediate DNA transfer *in vitro*. Barron *et al.* compared the surface activities of six different helical peptoid analogs of surfactant protein B (SP-B) to investigate the importance of mimicking its N-terminal insertion domain as well as its two arginine residues, both thought to be important for the peptide's proper function. A peptoid comprising a hydrophobic, helical insertion region with aromatic side chains showed more biomimetic surface activity than simpler peptoids, and even better activity, by comparison with natural lung surfactant, than SP-B_{1-25}. However, the substitution of lysine-like side chains for arginine-like side chains in the peptoid has little effect on biomimetic surface activity, indicating that interactions of the guanidino groups with lipids may not be critical for the function of these SP-B mimics [81]. In recent years, they have further demonstrated that peptoid-based SP-B mimics did generally mimic the surface activity of SP-B by substantially improving interfacial adsorption, maintaining a

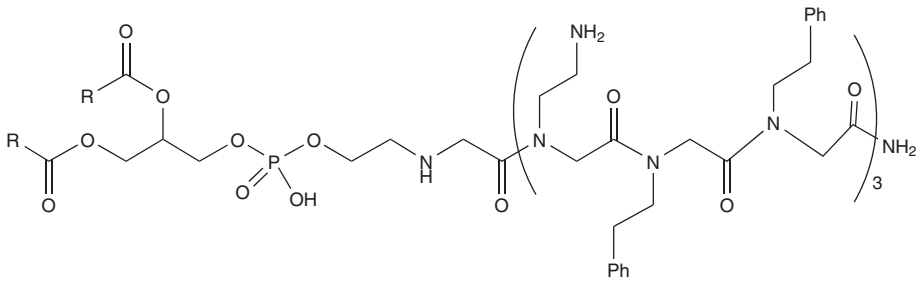

Figure 8. Chemical structure of lipids with peptoid head group.

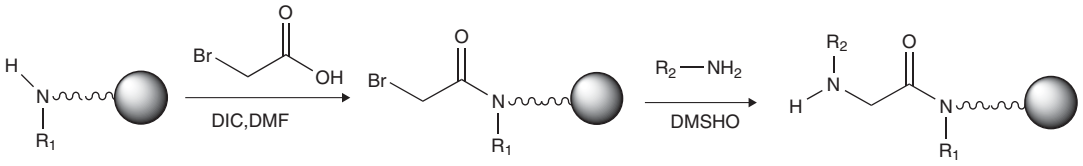

Figure 9. Scheme for the synthesis of peptoids (poly N-substituted glycines) on the solid phase using the submonomer method.

reduced surface tension and enhancing re-spreadability of lipid films [82].

6. Lipids with cholesterol hydrophobic group

Cholesterols are a kind of steroids and sterols. Their structure characteristics are that there is a β-hydroxyl on C-3, they often combine into sterol fat with fatty acid *in vivo*, there is a circuit double bond between C-5 and C-6, and there is an alkyl side chain containing eight carbon atoms on C-17. Gao and Huang [31] reported the synthesis and application of the cholesterol-based cationic lipid DC-Chol to deliver genes into mammalian cells. This cationic liposome reagent facilitated efficient DNA-mediated transfection in A431 human epidermoid carcinoma cells, A549 human lung carcinoma cells, L929 mouse fibroblast cells and YPT minipig primary endothelial cells. The activity was greater than that of a commercial reagent, Lipofectin, and was approximately four-fold less toxic than Lipofectin when assayed with A431 cells. Since then, considerable effort has been expended in the synthesis of steroidal cationic lipids owing to their potential applications in gene therapy [83]. As cholesterols have strong hydrophobic property and excellent biological affinity, they play the role of membrane fluidity regulator for liposomes reinforcing lipid bilayer membrane and reducing membrane flow, and thereby reducing the percolation ratio. Lipoplexes with high levels of cholesterol exhibited enhanced transfection *in vitro* and resistance to serum-induced aggregation [84]. Furthermore, cholesterol nano-domain formation was detected in lipoplexes with cholesterol contents above 52% (w/w). Considering that the cholesterol domain is neutral and separated from the positively charged lipid moiety that binds to anionic macromolecules (e.g., DNA, siRNA, serum proteins), it is potentially advantageous to utilize cholesterol domains as anchoring sites for ligands.

The molecular design of cholesterol-based peptide lipids utilized often involves the conjugation of a hydrophilic amine or polyamine group with a hydrophobic cholesterol motif, as shown in **Figure 10**. Sochanik *et al.* [25] coupled cholesterol with arginine sterically protected with one *N-tert*-butoxycarbonyl group (BOC) to synthesize a cationic lipid suitable for use as a DNA carrier in the presence of 10% sera. The liposomes showed superior activity *in vitro* transfection of B16 (F10) murine melanoma cells compared with that of liposomes made with other cationic derivatives of cholesterol. Choi *et al.* [24] have prepared and described DNA delivery agents composed of L-lysinamide, L-ornithinamide and cholesterol, which were designed to be readily susceptible to metabolic degradation after incorporation into animal cells. It was demonstrated that the cationic cholesterol derivatives were less toxic and more efficient in transfection than DC-Chol. The results for the biocompatible lipids suggest their potential use as general reagents for transfection of mammalian cells and *in vivo* applications for gene therapy.

7. Expert opinion

Among nonviral vectors, peptide cationic liposomes have drawn great attention, as they show low cytotoxicity, high transfection efficiency and wide range of transfection. Moreover, under physiological conditions, most amino groups are protonated in the process of carrying genes, and thereby with positive charge they could combine with negatively charged plasmid DNA to form liposomes/DNA complexes

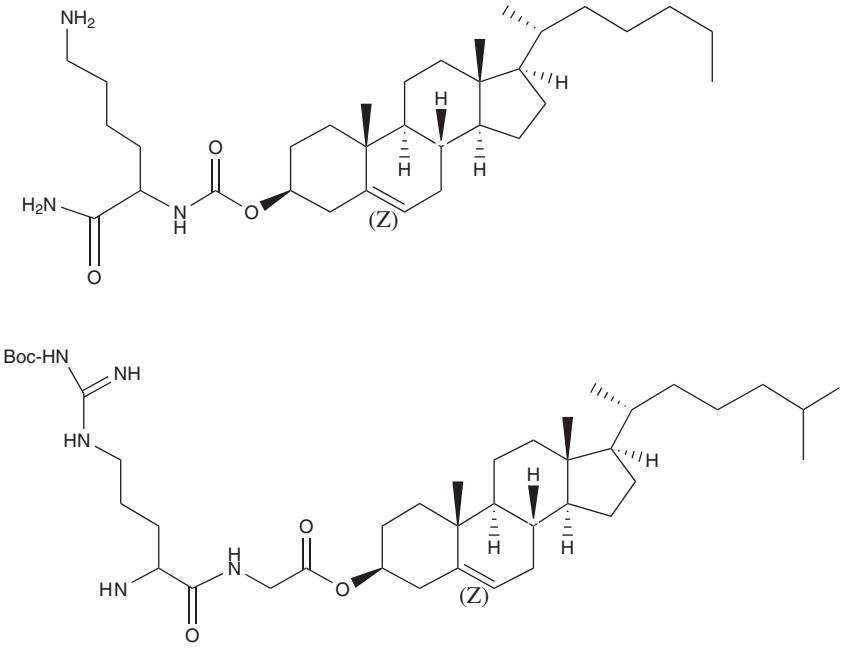

Figure 10. Chemical structure of peptide lipid with cholesterol hydrophobic group.

by electrostatic attraction. Since the first peptide cationic liposomes were prepared through a polycondensation reaction, many novel compounds of this kind have been obtained such as Gemini cationic lipids, lipitoids and cholesterol cationic lipids. The peptide-based Gemini lipids gain the attention, perhaps due to their superior surface-active properties and DNA-binding capabilities, which make them more suitable for the delivery of genes. The advantage of lipitoids may be the convenient synthesis to give strict control over the sequence of highly diverse monomers and then to generate extensive libraries of compounds thereof. Peptide-based cationic lipids with cholesterol group show strong hydrophobic property and excellent biological affinity, playing the role balancing membrane fluidity for liposomes reinforcing lipid bilayer membrane and membrane flow to make them more suitable for gene transfection.

Although peptide cationic liposomes have the potential to develop new paradigms in cellular gene delivery, application of peptide-based cationic liposomes for *in vivo* delivery of therapeutic DNA is still in its infancy and various areas of concern need to be critically addressed before significant solutions emerge. Therefore, the future research still needs to select suitable peptide heads and to investigate the surface modification of peptide cationic liposomes for facilitating the targeting. For example, Wang [85] constructed 'triply composite' consisting of plasmid, liposomes and the target ligand in order to improve the specificity of cationic liposomes to the target tissue. One of the most important reasons to use peptide cationic liposomes is their low cytotoxicity compared with conventional quaternary ammoniums, but it is not enough to put them into clinical applications; therefore, another trend is to continuously reduce the cytotoxicity through the design of head groups playing the key role of cytotoxicity. It has been stated that the combined utilization of cationic liposomes and other nonviral vectors may provide the most exciting solution for gene delivery, for many advantages could concentrate on the delivery systems. Therefore, new formulations containing peptide liposomes are deserved to be explored, such as the combination of peptide liposomes with viral carriers. Based on the above improvements and further research, peptide cationic liposomes will show endless potential in gene therapy.

Declaration of interest

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