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Cationic lipid–DNA complexes—lipoplexes—for gene transfer and therapy

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Abstract

Cationic lipid-mediated gene transfer and delivery still attract great attention of many gene therapy laboratories. From the point of view of the most important characteristics of lipoplex particles, e.g. its charge and size, we reviewed recent studies available. In general, the paper deals with non-viral systems of gene transfer into eukaryotic cell based on various lipids. Having usually less efficiency in gene transfer, lipid-based gene transfer vehicles (lipoplexes/genosomes) are characterized with certain advantages even over viral ones: they are less toxic and immunogenic, could be targetable and are easy for large-scale production, a size of transferred DNA being quite high. Conditions of DNA condensation during interactions with lipids are described. Results of the studies of mechanism of DNA–lipid complex interactions with the cell membrane and their transport into the nucleus are summarized. Dependence of efficiency of gene transfer on lipoplex structure and physical–chemical properties is reviewed. Advantages and disadvantages of different macromolecule complexes from the point of view of transfection efficiency, possibility of use in vivo, cytotoxicity and targeted gene transfer in certain organs and tissues are also discussed. Results of transfection of different cells using neutral, anion and cation liposomes are reviewed. The conclusion reached was that efficiency and specificity of gene transfer may grow considerably when mixed macromolecule lipid systems including polycations and glycolipids are used.

Keywords: DNA–(phospho)lipid complexes; Genosome; Lipoplex; In vitro and in vivo gene transfer; Cationic lipids; Polycations; Hydrophobic polycations; Non-viral targeted gene delivery

1. Introduction

Recently, the studies on gene delivery into eukaryotic cells by the use of non-viral vector-lipid-based macromolecular delivery systems have been experiencing a growing interest owing to the appearance of clinical protocols for gene therapy. Although the efficiency and specificity of such non-viral delivery systems are not yet very high, some of the problems concerning transfection methods are being successfully solved. To date, the transfection mediators that ensure effective and directed gene delivery into various cells have been created. Transfection of plasmid DNA is closely connected to the problem of condensation of its molecule since the plasmid is too large (13–15 kb) to effectively overcome the cellular membrane barrier. Besides, free DNA has to be protected from destruction by endogenous nucleases. Lastly, it is necessary to neutralize the negative charge on DNA. Almost all aspects of lipid–DNA complex structure and of gene transfer mechanism of non-viral systems based on them are connected to the electrostatic interactions and the decisive role of the charge, i.e. to bioelectrochemistry and even to the biodistribution of DNA–lipid complexes being dependent on particles' surface charge. It permits the authors to discuss the problems connected to the use of lipid–nucleic acid complexes in gene transfer and therapy in this paper. For DNA transport through the cellular membrane and further into the nucleus, a variety of complex-forming and condensing agents are used—such as polyvalent cations (Ca^{2+}, Mn^{2+}, $Co(NH_3)^{3+}$, La^{3+}), polycations (spermin, spermidin, histones, basic proteins, polyethylenimines), cationic polyelectrolytes (including polypeptides and dendrimers) and cationic liposomes. Condensation of DNA with these transfection

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mediators leads to its compaction with subsequent alterations in its superficial structure and hydrophobic properties and, most importantly, diminishes the size of a particle, thus promoting its penetration into the cell. DNA integrated into such a complex is protected from nuclease action [1–4]. Nowadays, the most widespread non-viral transfection systems for use in gene therapy are the lipid-based artificial macromolecular complexes. They involve cationic liposomes formed from diverse synthetic cationic and amphiphilic lipids as well as helper lipids—specifically, various natural and synthetic polycations (e.g. polylysine, polyethyleneimine), hydrophobic oligo- and polycations [5–13]. All the abovementioned non-viral vectors and systems accomplish the indirect, passive transport of functional genes into cells and tissues—except in cases where the macromolecular complexes are employed for receptor-mediated gene transfer [2,14]. The present work reviews the up-to-date amount of transfection data obtained by the use of natural and synthetic lipid-based polycations. Much attention is paid to the key points of the gene transfer mechanism using polycations and liposomes.

2. The mechanism of lipofection

Entry of the DNA–lipid complex into the cell may occur by two main pathways: (a) endocytosis with subsequent destruction of an endosome within the cell [15–18] and (b) direct fusion with cellular membrane [6] (Fig. 1). It was shown [19] that the major part of the complexes are internalized by endocytosis and only 2% of cells are transfected through direct complex-membrane fusion. Successful gene delivery by use of cationic liposomes requires the following conditions [20]: (1) condensation of DNA into the genosome and its protection from degradation by intracellular nucleases; (2) adhesion of DNA–lipid complex onto the cellular surface; (3) genosome internalization; (4) fusion of an internalized DNA–cationic liposome complex with the endosome membrane; (5) escape of DNA from the endosome; (6) entry of DNA into the nucleus followed by gene expression. Adhesion of the complex, containing the positively charged cationic liposomes onto the negatively charged outer membrane of the cell occurs through electrostatic interactions. Removal by the use of pronase of the negatively charged glycoprotein from the cellular membrane diminishes the transfection efficiency, whereas the treatment of cells with poly-L-lysine prior to transfection enhances it. This latter effect is probably connected to the formation of a protective layer, consisting of positively charged polypeptide residues on the negatively charged cellular surface, or to the enhanced adhesion of the complex [20].

3. Transgene expression in the nucleus

Transport of genetic construction into the nucleus is the key stage of efficiency of the gene delivery process. Experiments with a transfected (labeled [Au]DNA)-containing complex showed this complex to be localized mostly in the pericellular space [15]. The data obtained revealed that after lipofection, 98% of COS-1 cells contained plasmid in the cytoplasm, whereas only 10% of the cells expressed the reporter gene of β-galactosidase. Using the labeled complexes Felgner and Ringold [21] found that despite the adsorption by cells of 20–80% of plasmid DNA added, only less than 1% of the latter was detected in the nucleus. The ability of cells to express the transgene DNA is connected, among other factors, to its longevity in the nucleus. Normal human fibroblasts, which are difficult to transfect, were found to eliminate rapidly exogenous DNA

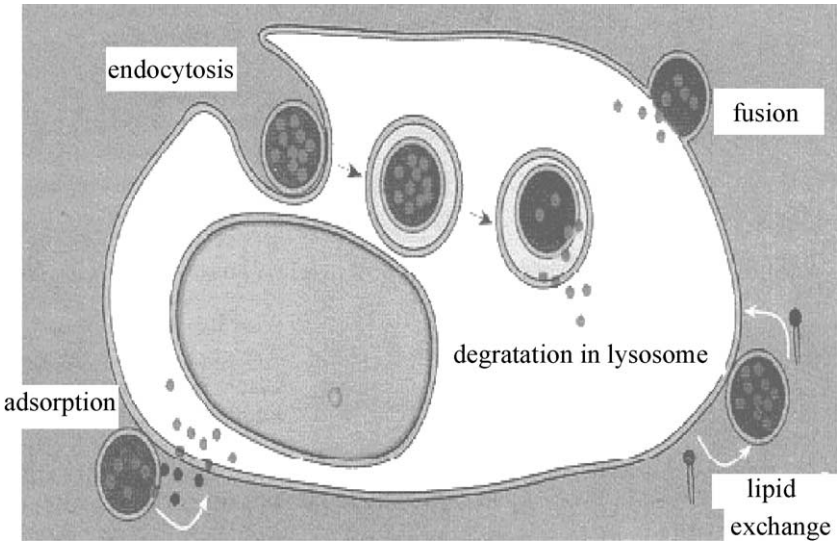

Fig. 1. Proposed mechanism for transfer of lipoplexes/genosomes to the nucleus (based on data: D. Lasic. Liposomes in Gene Transfer, CRC Press, Boca Raton, 1997 [4]).

from the nucleus. On the contrary, plasmid DNA retains stability in the nucleus of easily transfected HeLa cells for a very long time [22].

4. Anionic and neutral liposomes

As a rule, transfection by the use of anionic or neutral liposomes is not very efficient. On the whole, these liposomes are transfection-efficient when applied in vitro and much less efficient being applied in vivo. Experiments with usage of such glycolipid-complexed liposomes for targeted delivery of insulin-responsible genes into liver cells were described [14,23]. The construction of pH-sensitive, lymphoma cell-specific immunoliposomes was also described. However, transfection in both cases [24] was not very effective. Recently, we have proposed a non-cationic lipofection method based on the use of amphiphilic phospholipid vesicles in the presence of high concentration of bivalent metal ions (Ca, Mg) [1]. Phosphoryl groups of DNA within the neutral liposome-containing complexes are bridged with phosphoryl groups of phospholipids by divalent metal ions. The phenomenon of condensation of nucleic acids with amphiphilic phospholipids and formation of the phospholipid bilayer structures around the DNA/RNA in the presence of divalent metal cations was studied by a variety of biochemical [25–28] and biophysical [29–36] methods. Such complexes form a network consisting of nucleic acid and liposomes, bridged by metal(II) ions. Investigation of complex's structure by electron microscopic methods showed that DNA in such conditions is enwrapped into cylindrical (spaghetti-like) phospolipid bilayers [35,36], allowing it to retain stability in transfection conditions in vivo. Besides, transfection complexes, based on neutral phospholipids, are not subject to the major drawback of cationic liposomes—their ability to form complexes with DNA too tightly and hence preventing DNA release and its further way into the cell nucleus. It is worth to note that addition of anionic liposomes to the DNA–lipid complex leads to the rapid destabilization of a membrane and to DNA liberation [37].

Complexes of pH-sensitive liposomes, containing calcium ions and glycyrrhizin (GA) and the α-tocopherol ester of succinic acid (TS) (Fig. 2), were used for transfection of pSEAP and pQE-LacZ plasmids into various cell lines in vitro [38]. The glycyrrhizin structure consists of aglycon and of three residues of glucuronic acid (an oligosaccharide moiety which may also serve as an addressing group), since it is known that GA ensures tropicity of liposomes to the liver tissue. Components of liposomes, GA and TS, just like the cholesterol ester of TS used for this purpose earlier, carry the carboxy group residues, ensuring pH sensitivity. The efficiency of transfection into L929 cells with GA-containing amphiphilic liposomes in the presence of Ca ions (50 mM) was comparable with that of Ca–phosphate DNA precipitation. Usage of TS–liposomes led to the increase of

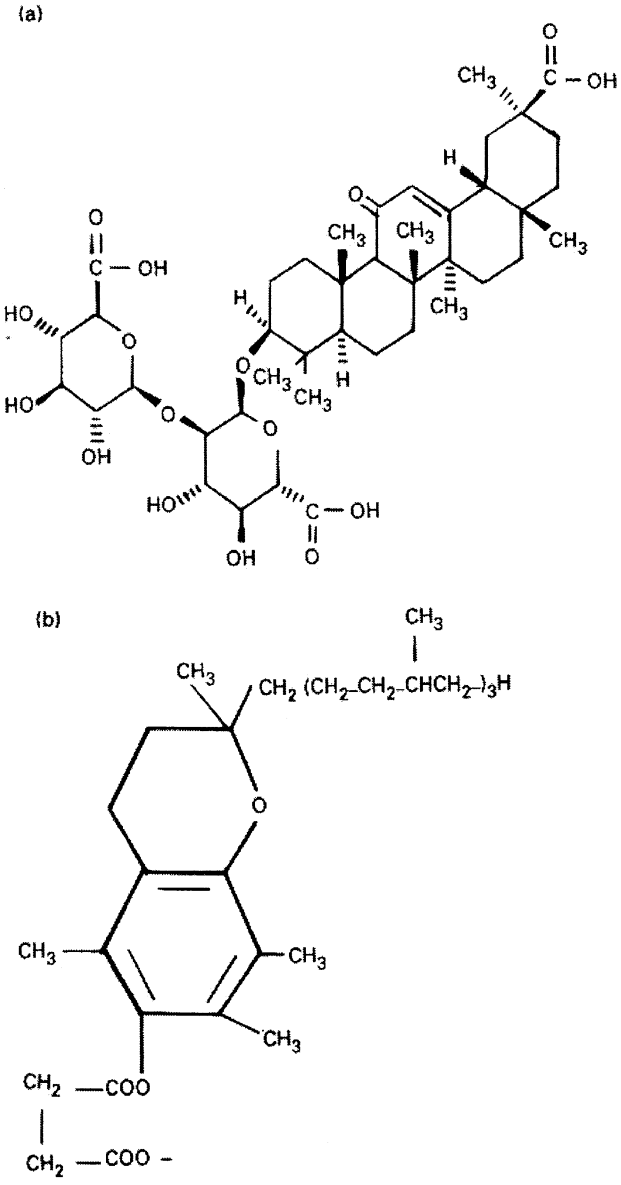

Fig. 2. Structure of new pH-sensitive agents [40]: glycyrrhyzin (a) and α-tocopherol ester of succinic acid (b).

transfection level compared to the one obtained for GA-containing liposomes. Comparison of transfection efficiency of amphiphilic and positively charged liposomes revealed insignificant differences in the efficiency of both gene delivery systems. Direct injections of amphiphilic liposomes' complexes with pQE-LacZ plasmid into mouse muscle also gave positive results [39]. Tissue distribution of [^{14}C]-DNA itself and corresponding PC/GA and PC/TS lipoplexes were examined following intraperitoneal administration of a 24-h postdose. The [^{14}C]-DNA itself was not detected in any organs at a 1.5-h postdose. The use of PC/GL or PC/TSA lipoplexes considerably changed the biodistribution of [^{14}C]-DNA in mice tissues. The maximal content of [^{14}C]-DNA for both types of lipoplexes was observed in intestine (50% dose equiv./g) and the spleen

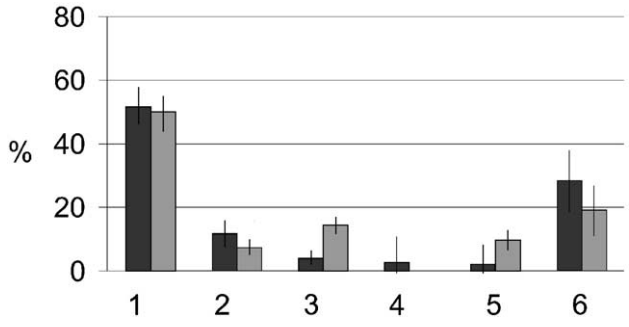

Fig. 3. Tissue distribution of PC/Chol liposomes containing glycyrrhizin (PC/GA) (solid column) or α-tocopherol ester of succinic acid (PC/TS) (open column) in ICR mice after (24 h) single intraperitoneal injection of 20 μg [^{14}C]-DNA in 200 μl of saline. The ordinate shows the percentage of total label detected in the organs observed. 1—spleen; 2—kidneys; 3—liver; 4—lungs; 5—heart; 6—intestine [40].

(30% dose equiv./g) (Fig. 3). The content of [^{14}C]-DNA in liver and kidneys was equal to 4% and 10% in the case of PC/GA-lipoplexes, and 15% and 6%, for PC/TS ones, respectively [40]. These data show the good possibility for application of such gene delivery systems; nevertheless, the efficiency of transfection using these systems is to be enhanced even more.

5. Cationic lipids and liposomes

Synthetic cationic lipids and liposomes are especially obtained on the basis of their perspective among the non-viral transfection systems (Fig. 4). The positive charge on the particle surface ensures their binding to the negatively charged cellular membranes. Cationic liposomes react spontaneously with the negatively charged DNA molecules (self-assembling system), forming complexes with 100% DNA molecules participating in the reaction [23]. It is shown that two processes are involved in the complex formation. A fast exothermic process is attributed to the electrostatic binding of DNA to the liposome surface [41]. A subsequent slower endothermic reaction is likely to be caused by the fusion of the two components and their rearrangement into a new structure. During this process, the homogenous and physi-

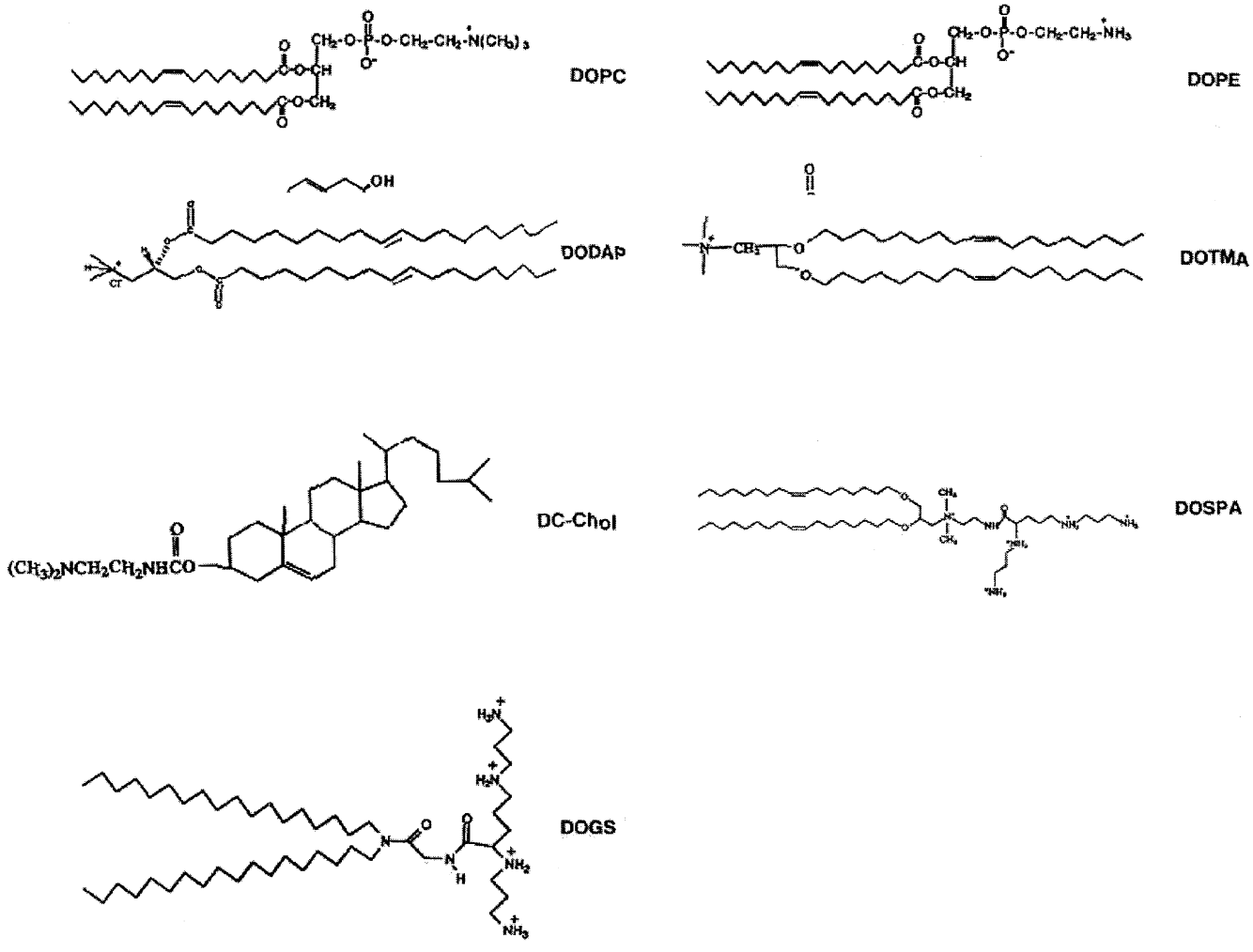

Fig. 4. Structure of cationic lipids designed for gene transfer and therapy.

cally stable suspensions are often formed [42]. Incorporation of only small amounts of anionic lipid into liposomes leads to DNA association with the inner surface of the liposomal membrane, which protects DNA against enzymatic degradation. To facilitate endocytosis, the incorporation of proteins such as anti-MNS-antibodies [43], transferrin [44] and Senday virus [45] into liposomes may be accomplished, which will allow for plasmid DNA penetration from the endosome into the cytoplasma, thereby avoiding degradation.

5.1. Synthetic cationic lipids and helper lipids

An example of such used mostly for transfection cationic lipids are dioctadecylamidoglicylspermin (DOGS or "transfectam") [11] or "lipofectin" [5,6] (Fig. 4). One of the first cationic lipids is dioleoxypropyl trimethylammonium chloride (DOTMA) [6] whose polar head group has the quaternary amine moiety. Thus, vesicles formed by DOTMA carry a positive charge on their surface. Cationic lipids may have monocationic head groups, such as DOTMA, dimyristooxypropyl dimethyl hydroxyethyl ammonium bromide (DMRI) [7], dioleoyloxy-3-(trimethylammonio)propane (DOTAP) [2], DC-Chol or polycationic head groups (DOSPA and DOGS) [2]. Dioleyl phosphatidylethanolamine (DOPE) is mostly used as a helper lipid for formation of liposomes from cationic lipids (excluding DOGS-based liposomes which cannot be used this way). In many cases, the equimolar mixture of a cationic lipid and DOPE ensures the optimally efficient transfection [7]. However, according to other sets of data, inclusion of DOPE into the genosome essentially lowers the transfection efficiency [42,46]. Liquid nature of a DOPE/lipid bilayer combined with the instability of such liposomes, promotes efficient binding and penetration of plasma proteins into the liposomal structure of plasma proteins [47]. For gene transfer in vivo, apart from DOTMA-based liposomes, other complexes (in equimolar ratios) are also used—such as dioctadecylamidoglicylspermidin (DLS)/DOPE [48], DOPE/DOTMA (1:1), DOPE/DOTAP (1:1) [46,49] and dimethyloctadecylammonium bromide (DDAB) and DOTAP with cholesterol (1:1) (mol/mol) [46,49]. Usage of neutral lipids such as cholesterol and its derivatives allows one to attain higher transfection levels in vivo [2,42,50]. Reliably higher expression in many organs was revealed upon application of cholesterol-containing liposomes [46,50] as compared to other liposomes. DOPE-containing liposomes, as well as various galactosylated cholesterol derivatives, exhibit low toxicity and high transfection efficiency with regard to human hepatoma cells, Hep G2. The efficiency enhancement is caused apparently by affinity to the asyaloglycoprotein receptor, specific for parenchymal liver cells [51].

Permanent search for new cationic lipids is conducted for creation of efficient gene delivery systems. Thus, liposomes, formed by *O*,*O*-ditetradecanoyl-*N*-(α-trimethylammonioacetyl)diethanolamine chloride (DC-6-14) and DOPE or cholesterol as helper lipid, exhibit high transfection efficiency with regard to disseminated peritoneal tumour cells. They are more efficient than commercial cationic liposomes such as "lipofectin", "lipofectACE" and "lipofectamine" [52]. New cationic lipids—rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride (**I**) (CLIP-1) and rac-[2(2,3-dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium bromide (**II**) (CLIP-6)—were used for transfection in vitro along with cationic lipid **III** (Fig. 5). These cationic lipids represent a set of cationic "rulers" with different linkers between hydrophobic and polar part: one chemical bond (**I**), six bonds (**II**) and nine bonds (**III**) [8]. Usually, it appears that the longer the linker part is, the higher is transfection activity for some cell lines used. Glycyrrhizin or the α-tocopherol ester of succinic acid [38] was included as pH-sensitive agents and fusogenes in the liposome composition. Cationic liposomes are the most probable alternative to viral delivery systems; the possibility of their application for gene delivery, both in vivo and in vitro, was verified in a series of experiments [5–8,21,49,50,53]. By using cationic liposomes, a relatively stable expression of transfected genes in various tissues was achieved [48,49,54]. The major disadvantage of cationic liposomes as a gene delivery system is their much lower transfection efficiency compared to viral vector systems.

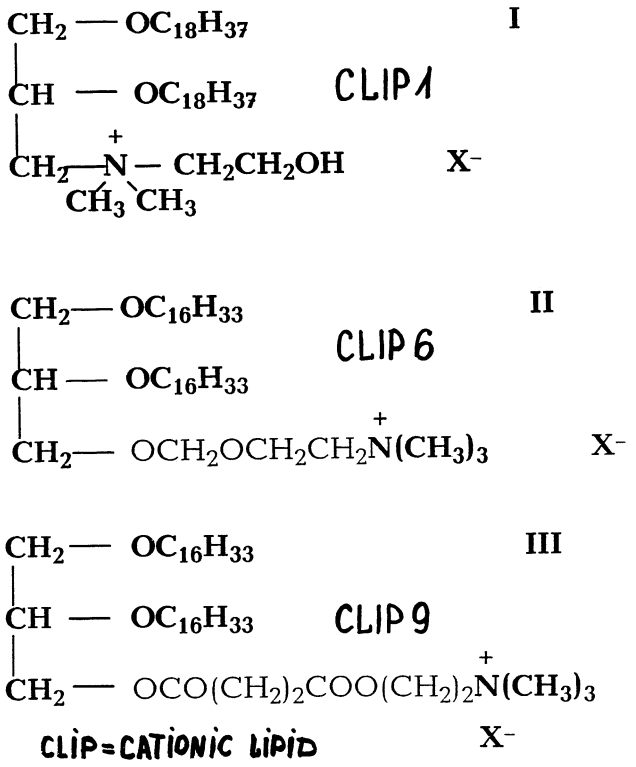

Fig. 5. Structure of new cationic lipid rulers [8]: *rac*-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride, (**I**) (one chemical bond between hydrophobic and polar parts of the molecule); *rac*-[2(2,3-dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium, (**II**) (six chemical bonds); rac-[2(2,3-dihexadecyloxypropyl-oxysuccinyloxy)ethyl]-trimethylammonium (**III**) (nine chemical bonds).

The limitations of cationic liposome usage for transfection purposes (as a gene therapy tool) are closely connected to a short lifetime of the complexes, as well as to their inactivation by serum proteins and toxicity of cationic lipids in high concentrations. Taking into account an important role of cholesterol in efficient gene transfer, a series of oligocations—oligo(ethylen)propylenimines—containing one, two or three cholesteryl moieties (Fig. 6) [13], provide a possibility to vary hydrophobicity/hydrophilicity ratio inside the same group of transfection agents, which could be important to provide targeted delivery of therapeutic genes. They represent a new group of transfection mediators with high transfection efficiency [3,55,71,78].

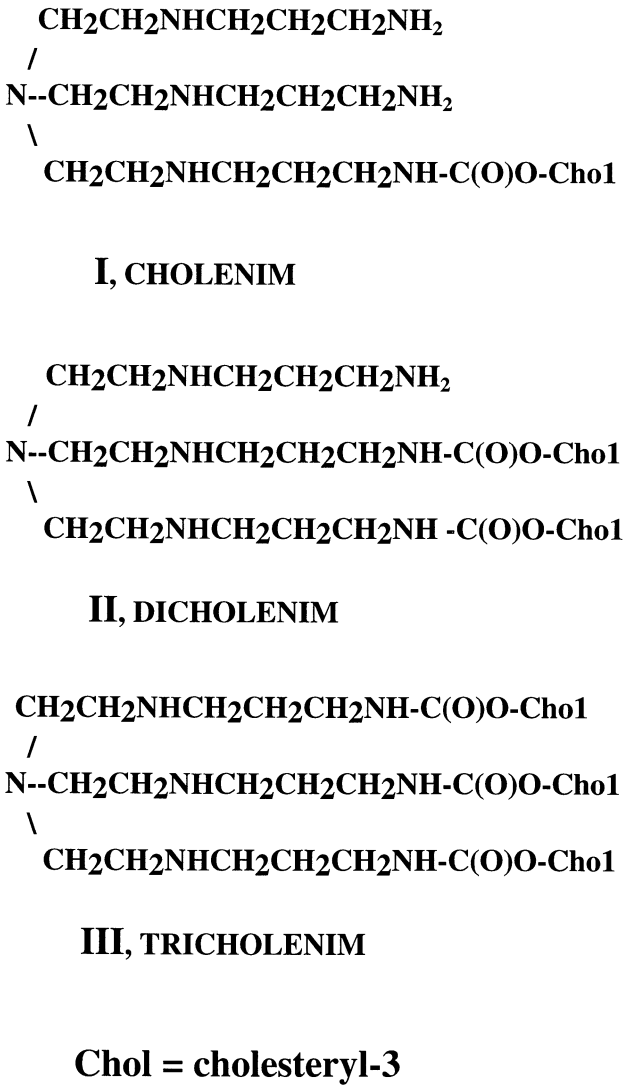

Fig. 6. Structure of hydrophobic oligocations-3-cholesteryl derivatives of co-oligomer hexaethylen/propylen/imine: I—(tris-[2-*N*-(3-aminopropyl)aminoethyl]amine monocholesteryl acylformiate, CHOLENIM; II—(tris-[2-*N*-(3-aminopropyl) aminoethyl]amine dicholesteryl acylformiate, DICHOLENIM; III—tris-[2-*N*-(3-aminopropyl)aminoethyl]amine tricholesteryl acylformiate, TRICHOLENIM (Vlassov et al., 1997) [13].

5.2. Possibilities for increasing gene transfer efficacy

At present, the role of various factors affecting transfection efficiency remains virtually unclear. However, modification of methods for cationic liposome presentation and for incorporation of various proteins, peptides and other compounds into their structure allows us to overcome some difficulties connected to liposomal systems' disadvantages in gene transfer. Thus, the toxicity of DNA/liposome complexes (in the case of DOSMA/DOPE) was essentially lowered while their stability was enhanced by the use of 1% octylglucoside for complex preparation [56]. Cationic liposomes containing the cholesterol derivative cholesteroyl-3β-carboxyamidoethylen-*N*-hydroxyethylamine [57] are characterized by rather high transfection efficiency even in the presence of serum proteins. This property makes possible the application of such systems for gene delivery in vivo. Reliably higher transgene expression was registered in many organs upon usage of liposomes containing DODAB/Chol, DOIC/Chol and DOTAP/Chol—as compared to those prepared on the basis of neutral lipids [46,50]. According to Song and Liu [58], the increase in the lipid/DNA ratio has a positive effect on the transfection efficiency in vivo. The authors believe that free liposomes, mixed with the DNA–lipid complex, protect DNA from degradation and thus increase the duration of transgene expression. Suppression of genosomes' binding by plasma proteins may be attained by increasing the positive charge of the complex.

Polylysine (as polycation) is able to condense DNA efficiently—which may probably be explained by high density of its molecule's charge [59]. A vector system containing poly-L-lysine and DC-Chol/DOPE liposomes was designated as lipoplex (LPDI) [60]. This gene transfer system displayed a much higher transfection efficiency than the same liposomes without polylysine did. Electron microscopic studies have shown that LPDI are spherical particles of 50–75 nm, coated with a dense layer of a cortex-like substance, which is most probably nothing else but the polylysine-condensed DNA. High efficiency of transfection seems to be connected with small complex size; however, some authors think that transfection efficiency of cationic liposomes is size independent [4].

5.3. Role of structural characteristics of lipoplexes

The correlation between such parameters as the genosomes' form, structure and type of interaction with the cell, efficiency of gene delivery and expression level have been studied insufficiently. Using the electron cryomicroscopy method of high resolution, several types of complexes differing in their form were revealed. DOTAP- and DOTMA/Chol-based lyposomes form spherical genosomes, with DNA being condensed within them, whereas in the case of DODAP/Chol, DODAB/DOPE, DOIC/Chol, DMEPC/Chol, DMEPC/DOPE and DOTMA/DOPE genosomes have a somewhat different form [2]. In genosomes

containing the liposomal form based on DOTAP and cholesterol, DNA condenses on the inner side of a concave liposome between the two lipid layers [46]. This DNA state ensures its reliable protection against degradation and efficient transgene expression as well.

Oberle et al. [61] studied the interaction between plasmid DNA and cationic amphiphiles, using a monolayer technique, and examined the complex assembly by atomic force microscopy. The data reveal a three-step mechanism for complex formation. In a first step, the plasmid, interacting with the monolayer, displays a strong multilamellar fashion. Subsequently, individual plasmids enwrap themselves with amphiphile molecules in a multilamellar fashion. The size of the complex formed is determined by the supercoiled size of the plasmid, and calculations reveal that the plasmid can be surrounded by three to five bilayers of the amphiphile.

Sternberg et al. [62] showed that the requirements for the complex structure are different for in vitro as opposed to in vivo transfection. In the former case, high transfection efficiency is characteristic of genosomes with hexagonal packing of lipids, while in the latter case, most of the transfection efficiency is shown by highly stabilized complexes, in particular, those that have some projections (mappin structure). The essential requirement for efficient in vivo transfection is the small liposomal size (40–80 nm), while for in vitro transfected complexes, 200–400 nm is the optimal size.

5.4. Role of different stages of lipoplexes' internalization

Inhibition of endocytosis by citocholasine B suppresses transfection [20]. Chloroquine is a substance capable of enhancing lysosomal and endosomal pH and of inhibiting endosomes' ripening. The character of its influence on transfection efficiency allows one to elucidate, in some cases, the transfection mechanism for complex delivery, although the results obtained by different research groups are often interpreted differently. The study [7] explains the decreasing transfection efficiency of cationic liposomes containing DOPE and the cationic lipid (DMRI) after chloroquine addition by its toxicity. However, chloroquine was reported to enhance the efficiency of transfection after the substitution of DORI for DMRI in liposomal composition. Legendre and Szoka [63] also described the increase in transfection efficiency after chloroquine addition in the case of DOTMA/DOPE liposomes. Chloroquine has a negative influence on carcinoma cell transfection by liposomes, consisting of DC-Chol/DOPE. It is believed that some liposomes are released into the cytoplasm from the early endosomes while other liposomes are able to do so at the stage of late endosomes. Chloroquine, by inhibiting the endosomes' ripening, exerts a negative influence on transfection effected by those liposomes interacting with late endosomes [7,63,64]. It is worthy to note that the influence of chloroquine on transfection is different for various cell types [65].

Transfection by cationic liposomes is strongly dependent not only on their absorption by the cells, but also on the ability of lipids to be the part of a liposomal composition to destabilize endosomal membranes [66]. The efficiency of transfection by use of cationic liposomes is also increased after the incorporation of helper lipids such as DOPE into the liposome [7,67]. It is likely that the role of helper lipids is in triggering the mechanism of liposomal membrane/cellular membrane coupling—being a fusogen, the helper is able to do so. Then, after penetration of the transfection complex into the cytoplasm, the helper lipid destabilizes the endosomal membrane and allows the DNA to escape much earlier than it could be subjected to degradation. Fusogenic peptides are peptides such as the N-terminal peptide of the HA2 hemagglutinin of a flu virus or the pH-sensitive synthetic GALA peptide [68]. Experiments on transfection of NIH3T3, COS-7 and HeLa cells, by the use of cationic liposomes containing DOPE or cholesterol derivative, have demonstrated that DNA release from the endosome occurs following the fusion of liposomal/endosomal membranes at pH 5.0 [69]. Cholesterol is able not only to enhance the liposomes' stability in the blood flow upon transfection in vivo, but also to increase their biodegradation and subsequent DNA release [22].

The +/− charge ratio and the zeta potential is of great importance for the interaction of the cationic lipid–DNA complex with the cell. With the +/− ratio approaching 1, the zeta potential decreases and the aggregation of the complexes takes place, indicating that the binding of liposomes and lipoplexes to the cellular membrane occurs through electrostatic interactions [70]. Fusion process depends, to a great extent, on the complex's lipid composition, which seems to determine the sterically preferable lipid–molecule interactions on the cellular surface [70]. A complex consisting of DNA, cationic liposomes (DOTAP-DOPE) and transferrin with the total negative charge proved to be more efficient for gene transfer than the analogous positively charged complexes. For destabilization of endosomal membranes, a pH-sensitive peptide (GALA) was incorporated into the complex composition [68]. Such a quaternary complex is characterized by a high degree of transfection and by low toxicity. Possessing the total negative charge, it does not interact with serum proteins and therefore may be successfully used for in vivo transfection.

6. Macromolecular systems for targeted gene transfer

Since cationic liposomes are unable to exhibit specificity to certain cell types while interacting (mostly electrostatically) with the cellular membrane by themselves, a series of ligands able to target the gene transfer were employed. The incorporation of a glycolipid into the liposomal composition determines its tropicity to hepatocytes and to endothelial liver cells [14]. Transformed cells express much greater amounts of lectins (cellular membrane proteins) which are

involved in the endocytosis and intercellular interaction and bind specific glyco-conjugates. That is why using liposomes carrying carbohydrate ligands one can effect direct gene transfer into tumor cells [2].

Carbohydrate-conjugated polycations or lipids are probably the most effective transfection system for receptor-mediated transfer. We have used a cationic glycolipid containing a glucose residue between cationic and hydrophobic parts of lipid molecule (GLYCOCLIP, Fig. 7) for transfection as well as the liposomes prepared on its basis [71]. The results show that incorporation of a glucose residue into the cationic lipid enhances the transfection of CHO cells by the pLuc gene. Mixed liposomes (GLYCOCLIP/CHOLENIM/DOPE) containing a monocholesterol derivative of hexaethyleneimine display a transfection level 10-folds higher than GLYCOCLIP/DOPE does. It appears that cationic glycolipids represent a new class of compounds comparable in efficiency to commercial preparations of "DOSPER" type and may be used for gene transfer in vivo [71]. Liposome-bound antibodies may interact specifically with superficial antigen, thereby effecting the absorption (through endocytosis) of liposomes by the cells expressing these antigens. Usage of monoclonal antibodies to cellular surface antigens increases substantially the efficiency of targeted delivery of various pharmaceutical drugs and liposomal gene medicines into the cells of various tumors [72–75].

Because all vector systems discussed in this article represent artificial macromolecular complexes, it is important to know their immunogenic properties. It was shown that a number of synthetic cationic lipids, polyethyleneimine, polylysine and transferrin–polylysine activate the complement system to varying extents [76]. Strong complement activation is seen with long-chain polylysines, the dendrimer (of the fifth generation, PAMAM), polyethylenimine and DOGS. The other cationic lipids appeared to be weak activators of the complement system. The degree of complement activation by DNA complexes is strongly dependent on the ratio of polycation and DNA (charge ratio). To a lesser degree, charge ratio also influences complement activation by monovalent cationic lipid–DNA complexes [76]. The authors of Ref. [77] showed that repeated injections of plasmid complex with the anti-secretory component of Fab antibody evoked a humoral immune response against the heterogous Fab portion of the complex which is associated with reducing gene transfer efficacy. The effect of pCMV-SPORT-LacZ plasmid, negatively

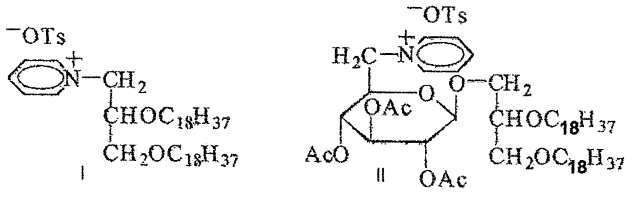

Fig. 7. Structure of cationic lipid and cationic glycolipid, containing glucose moiety as a linker [71].

charged cardiolipin (CL), neutral phosphatidylcholine (PC) and their lipoplexes on the complement system was studied using a simple method developed to detect the ability of effectors (lipoplexes) to activate the complement system in an antibody-independent manner to serve as acceptors of nascent C4b and to inhibit formation of the key enzyme of the complement, C3-convertase [78]. It was revealed that PC vesicles did not affect the complement system, while CL vesicles manifested low activation. The influence of plasmid DNA and PC lipoplex on the complement system was very low. Weak activation of the complement system with CL lipoplex, and even weaker for the PC lipoplex, testifies to the use of neutral and positively charged lipoplexes preferably in gene therapy protocols [78].

A number of high-quality articles, reviews and comments devoted to a variety of aspects of gene transfer and therapy were published in the period of processing this paper [79–113], including two special issues of Russian biomedical journals [112,113].

7. Conclusions

Despite many advantages of non-viral gene transfer systems, particularly of cationic lipids and liposomes created on their basis, these systems cannot replace completely viral vectors in gene therapy protocols. First, their transgene expression level is not so high as that of non-viral gene transfer systems, both in vitro and in vivo; this is mostly connected to complexity of the mechanism for penetration of genosome-integrated exogenous DNA into the cell nucleus. In cases where the liposomes are used for gene transfer, the problem of structural polymorphism of lipid–DNA complexes inevitably emerges; the differences in transfection efficiency of various liposome preparations are probably conditioned by heterogenity of these complexes. This warrants further studies of DNA condensation and the physico-chemical properties of the complexes formed with the view of obtaining the most efficient and quite stable gene transfer systems giving reproducible transfection results. From this viewpoint, the most promising is the elaboration of such non-viral gene transfer systems that enable to control DNA state in genosomes. Substantial enhancement of transfection efficiency by using combined systems of viral elements plus polycations [114,115], as well as mixed macromolecular systems involving polycations and glycolipids, explains the keen interest in such multicomponent gene transfer systems. The efficiency of directed gene transfer into certain tissues and organs in vivo may be enhanced by the use of immunoliposomes and ligands specific to certain proteins of cellular membrane.

The following features which are considered in articles reviewed can be decisive for transfection and targeting efficacy.

(a) Cationic lipid–DNA complexes represent self-assembling systems, supercoiled DNA being condensed inside

spherical particles (50–200 nm) sandwiched between lipid bilayers and being a promoter of supramolecular organization characterized by ordered multilamellar domains (with regular spacing 70–80 Å) to result in successful gene transfer [35,36,62,116].

(b) The bigger the size of lipoplex/genosome particles, the more the transfection level in vitro, and the less in vivo, and vice versa, with optimal size of these lipoplex particles being 70–200 nm.

(c) High transfection efficiency in vitro is characteristic of genosomes with hexagonal packing of lipids (200–400 Å), while in vivo, most of the transfection efficiency is shown by highly stabilized complexes (40–80 Å), in particular, those that have some projections (map-pin structure) [62].

(d) Lipoplexes usually have a high positive surface potential (180–240 mV) and pH value (10–11.5) [117]; the charge of lipoplex particles should not be too positive (and stable), otherwise, lipoplex will not dissociate and will not reach the nucleus; the +/− charge ratio and the zeta potential are of great importance for the interaction of the cationic lipid–DNA complex with the cell and should be above 1.

(e) In many cases, the equimolar mixture of a cationic lipid and DOPE ensures the optimally efficient transfection [7].

(f) Usage of neutral lipids such as cholesterol and its derivatives allows one to attain higher transfection levels in vivo [2,42,50]; cholesterol is able not only to enhance the liposomes' stability in the blood flow upon transfection in vivo, but also to increase their biodegradation and subsequent DNA release [22].

(g) The highest lipofection levels are obtained under conditions of lipoplex instability, when DNA is partially dehydrated and has C- or partial Psi-structure [117].

(h) For systemic gene delivery and expression in tumor, it is important to optimize both parameters: cationic lipid-to-colipid (DOPE or cholesterol) molar ratio and lipid-to-plasmid charge ratio, the values being, correspondingly, 4:1 and 3:1 [118].

(i) There is an effect of passive targeting of particles of certain size ($\sim 80 \pm 20$ nm), which permit them to be accumulated in malignant tissues; this effect is due to inner diameter of tumor vessels.

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