Biological and materials properties of various cholesterol based systems

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Abstract | Liposomes composed of cationic lipids have become very popular gene delivery vehicles. A great deal of research is being pursued to make efficient vectors by varying their molecular architecture. Cholesterol being ubiquitous component in most of the animal cell membranes is increasingly being used as a hydrophobic segment of synthetic cationic lipids. In this review we describe various cholesterol based cationic lipids and focus on the effect of modifying various structural segments like linker and the head group of the cationic lipids on gene transfection efficiency with a special emphasis on the importance of ether linkage between cholesteryl backbone and the polar head group. Interaction of cationic cholesteryl lipids with dipalmitylphosphatidycholine membranes is also discussed here. Apart from cholesterol being an attractive scaffold in the drug/gene delivery vehicles, certain cholesteryl derivatives have also been shown to be attractive room temperature liquid-crystalline materials.

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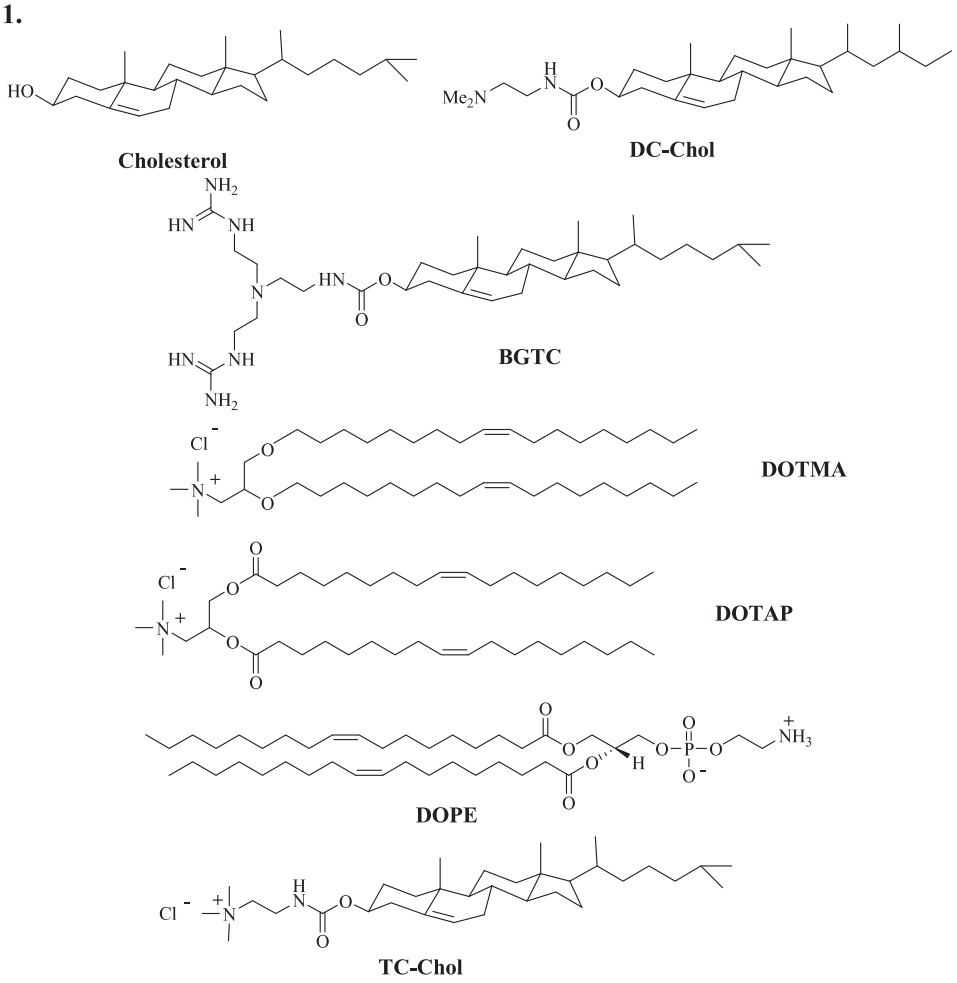
Introduction

Cationic lipids are currently gaining importance because of their promise in gene therapy. Due to their opposite surface charge, they can form a complex with the negatively charged DNA and help in favorable uptake of the resulting lipoplexes by the cell. A typical cationic lipid may be visualized to have a hydrophobic segment, specific backbone, headgroup and a linkage region. The most commonly used cationic lipids can be divided into two major groups based on the molecular structure of their hydrophobic moiety. In one group, this moiety is consist of a long hydrocarbon chain while in the other, it is based on a steroid (cholesterol). Among the many cationic lipids of the first variety developed in the past, the most commonly used molecules include DOTMA (first lipid reported by Felgner *et al.*, 1987) and DOTAP (Leventis and Silvius, 1990) (Chart 1).[1,2] Currently several research groups are however, focusing on cytofectins based on a steroid backbone, especially cholesterol. The most commonly used cationic lipid with the cholesterol moiety as the hydrophobic anchor is DC-Chol, the first steroid-based lipid reported by Gao and Huang (Chart 1).[3] Huang and Gao showed that DC-Chol was better than the commercially available Lipofectin in terms of lower toxicity and greater transfection efficiency. Although there is variation in their transfection efficiency, both types of cationic lipids are active in transfecting many types of cells *in vivo* (Gao and Huang, 1995).[4] However, liposomes prepared with these two groups of cationic lipids have shown different activities *in vivo* in systematic transfection of the lung endothelium. Since the first report of a cationic lipid (DOTMA) by Felgner *et al.*, an increasing number of new cationic lipids based have been synthesized with modifications at headgroup and other regions of the lipid molecules. Compared to a number of published studies that reported the systematic transfection

CHART 1.

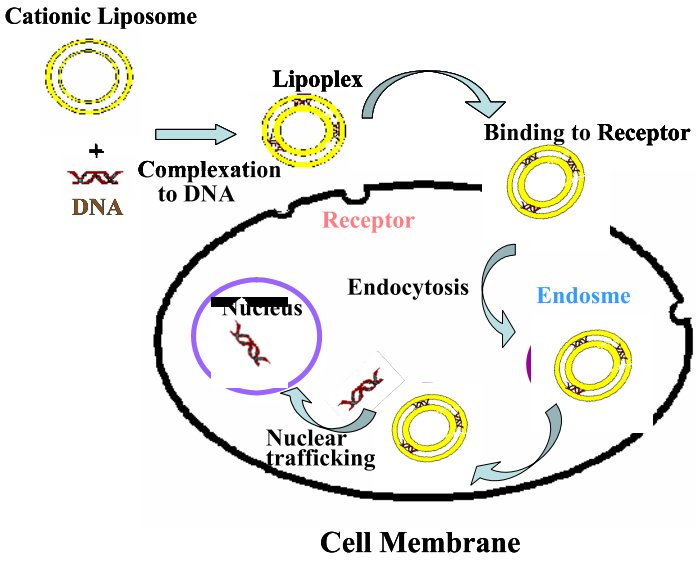


efficiency of long hydrocarbon chain-based cationic lipids,[5–11] reports of the success of cholesterol-based cationic lipids in this area remained few until recently. Using cholesterol-based cationic liposomes and the luciferase gene as a reporter, it was found that, under optimal conditions, the level of gene expression in the lungs of animals transfected by DNA complexed with either DC-Chol or TC-Chol (trimethylaminoethane carbamoyl cholesterol) liposomes is much lower than that of complexes made with either DOTMA or DOTAP (Chart 1). These data suggested that cholesterol-based cationic liposomes were less active in the systematic transfection of the lung endothelium.[12] This prompted the design of newer cholesterol based cationic lipids for achieving efficient transfection. Prominent among them were with polyamine variants of DC-Chol, having bis-guanidinium unit in the headgroup (BGTC) (Chart 1).[13]

Leventis and Silvius (1990) investigated, in addition to three different derivatives of DOTAP (1,2-dioleoyloxy-3-[trimethylammonio]-propane), quaternary ammonium ion attached to "Cholesterol" possessing two different spacers, five and seven atoms long, with different spacer segment hydrophobicity.[2] Lipid mixing upon interaction of such cationic lipid aggregates with phosphatidylcholine/phosphatidylserine vesicles did not show any apparent correlation with the transfection activity. Huang and colleagues (Farhood *et al.*, 1992) also examined several different cholesterol derivatives.[14] From their study, it was obvious that for subsequent studies and applications, tertiary DC-Chol which consists of a hydrolysable carbamoyl bond, a spacer of three atoms, and a tertiary amino group would be worthwhile. The highest stability of the formulation formed was observed with the mixture of DC-chol and DOPE at 3:2 molar ratio. This aggregate also showed high transfection efficiency in epithelial cells, and in lymphocytes. These observations are consistent with the well-known dependence of transfection activity on cell lines as well as on cell-plating density.

Polyethylene glycol (PEG) linked lipids have been developed to achieve stealth liposomes which have increased bioavailability and longer circulation times in sterically stabilized systems PEG-cholesterol however, did not provide an efficient formulation because cholesterol is not a very good anchor to retain polymer-lipid in the membrane for prolonged

Figure 1: Schematic representation of the mechanism of DNA delivery into the cells with synthetic lipid vectors.



times. One would anticipate that the fate of the cholesterol anchor would be similar especially if linked to multivalent cationic charges. While this may not be a problem for in vitro or direct, localized applications, it could certainly reduce association of such molecules in bilayers upon systematic application. What happens to the complexed lipid molecules is still not known, but it is likely that they are associated with complex more strongly. The excess of cationic lipids, however, is toxic and is likely to increase membrane permeabilization. In most cases, however, this excess can be removed by centrifugation which can significantly reduce the toxicity of the preparation.

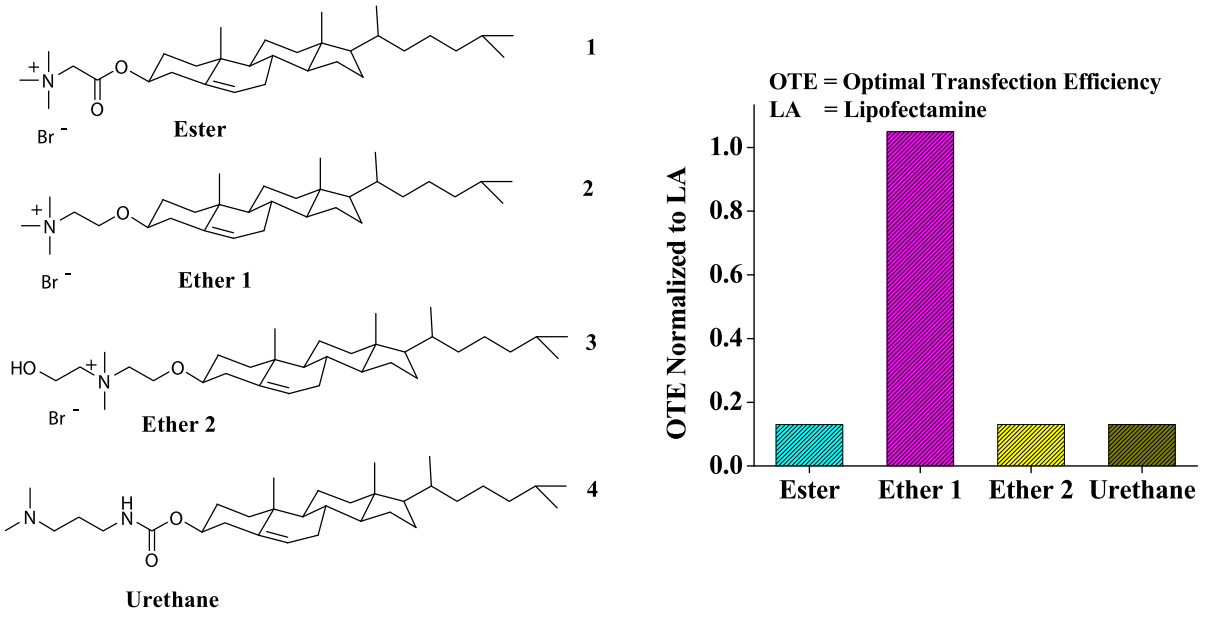
Mechanisms of DNA delivery by synthetic vectors

A central aim of DNA delivery by synthetic lipid based vectors is that DNA molecules can only be delivered into a cell when they are converted into a lipoplex form. This appears to be true for practically all of the lipid vectors that have been synthesized for gene transfection so far. The synthetic materials such as calcium phosphate, DEAE-dextran, cationic lipids and cationic polymers are all capable of forming particles in nanometer dimension upon complexation with DNA. With the exception of the calcium phosphate method that forms calcium-phosphate-DNA precipitates; other synthetic compounds form complexes with DNA through electrostatic interaction between DNA and the synthetic vectors (*i.e.*, lipoplexes). Once complexed with a lipidic vector, DNA molecules are protected against nuclease-mediated degradation. To accomplish DNA delivery, these complexes need to (1) bind to the cell surface; (2) cross the plasma membrane; (3) release DNA into the cytoplasm; and finally, (4) transport the DNA into the nucleus (Fig. 1).

Basic structural features of any cationic lipid

A cationic lipid is a positively charged amphiphile, which generally contains the three following structural segments: (1) a hydrophilic headgroup which is positively charged, either *via* the protonation of one (monovalent lipid) or several (multivalent lipid) amino groups or quarternized ammonium group; (2) a hydrophobic portion composed of a steroid or of alkyl chains (saturated or unsaturated); and (3) a linker region (connecting the cationic headgroup with the hydrophobic anchor) whose nature and the length may impact on the stability, cytotoxicity and the biodegradability of the vector. Since the initial validation period, progress has been made in the design of each of these moieties. The choice of the cationic headgroups has expanded into the use of natural architectures and functional groups with recognized DNA binding modes as well as non-amino-based cationic moieties. Modifications of the hydrophobic domain have shown that optimal vector structure is often dependent on this moiety, which falls into various structural subclasses and variants. Finally, labile linkers have been introduced which

Figure 2: Molecular structure of the cationic cholesterol-based amphiphiles with different linker groups (left) and their transfection efficiencies at lipid/DOPE ratio 1:1 (right).



are sensitive to various biological stimuli, inducing DNA release at defined time points during the intracellular trafficking of the DNA bound lipoplex.

Design of cholesterol-based cationic lipids

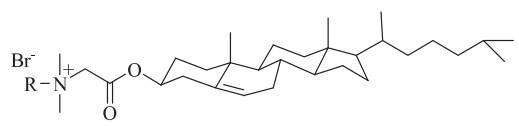
The advantage of cationic cholesterol derivatives over traditional glycerol-based amphiphiles is that the latter are normally used in conjunction with a helper lipid like DOPE to enhance their efficiency.[15] This limits their shelf-life as these formulations which tend to phase separate gradually over a period of time. Also, the 3β-OH of cholesterol can be functionalized which facilitated structural modifications at head group and linker. The transfection efficiency of this class of molecules is highly dependent on the structure of cholesterol monomer, although the mechanism of DNA transfection by them is not known.

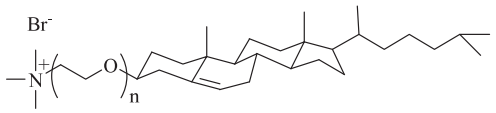
Cholesterol has a polar hydroxyl group and a non-polar steroidal ring system with an isopentyl chain. This makes it weakly amphiphathic in nature and due to this it forms an aggregate in aqueous media. Appending its hydrophobic residue with a polar moiety at 3β-OH should result molecules with improved amphiphatic properties which can form thermally stable membranes and indeed it was found to be so.[16–20]

Towards this end, we synthesized three cationic cholesterol derivatives with different linkage types, viz. an ester (**1**), ether (**2**) and urethane (**4**) between the cationic headgroup and the cholesteryl backbone (Fig. 2).[21] To study the effect of hydration at the headgroup, a hydroxyethyl group was also incorporated. These were mixed with DOPE for the preparation of liposomes to induce liposome-mediated gene transfection. When the optimal transfection efficacies for a particular lipid were compared, lipids with ester and urethane linkage showed much less transfection efficiency compared to their ether counterpart. In fact replacement of the urethane linkage with an ether linkage led to a 6-fold increase in transfection efficiency. Amphiphiles with ether links such as cholest-5-en-3β-oxyethane-*N*,*N*-dimethyl-*N*-2-hydroxyethyl ammonium bromide (**3**) and cholest-5-en-3β-oxyethane-*N*,*N*,*N*-trimethylammonium bromide (**2**) showed transfection efficiencies considerably greater than commercially available gene transfer reagent, Lipofectin. Lipid 1, is considerably more efficient than the commercially available formulations of Lipofectamine and Lipofectin, especially in the presence of serum. In addition, (**2**) was able to transfect efficiently without a helper lipid like DOPE which increased its usefulness significantly.

Based on these results, we then undertook a detailed study by synthesizing different cationic cholesterol derivatives with different linkage types between the cationic headgroup and the cholesteryl backbone for their utilization in transfection through various cell lines (Fig. 3).[22] Also, varying lengths of oligo-oxyethylene glycol (OEG) units

Figure 3: Chemical structures of cholesterol-based cationic lipids with different linkers and head groups.





5a: R = CH_3
5b: R = CH_2CH_2OH
5c: R = $(CH_2CH_2O)_2H$
5d: R = $(CH_2CH_2O)_4H$
7a: R = cis-$(CH_2)_8CH{=}CH(CH_2)_7CH_3$
7c: R = cis-$(CH_2\text{-}CH_2O)_4CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$

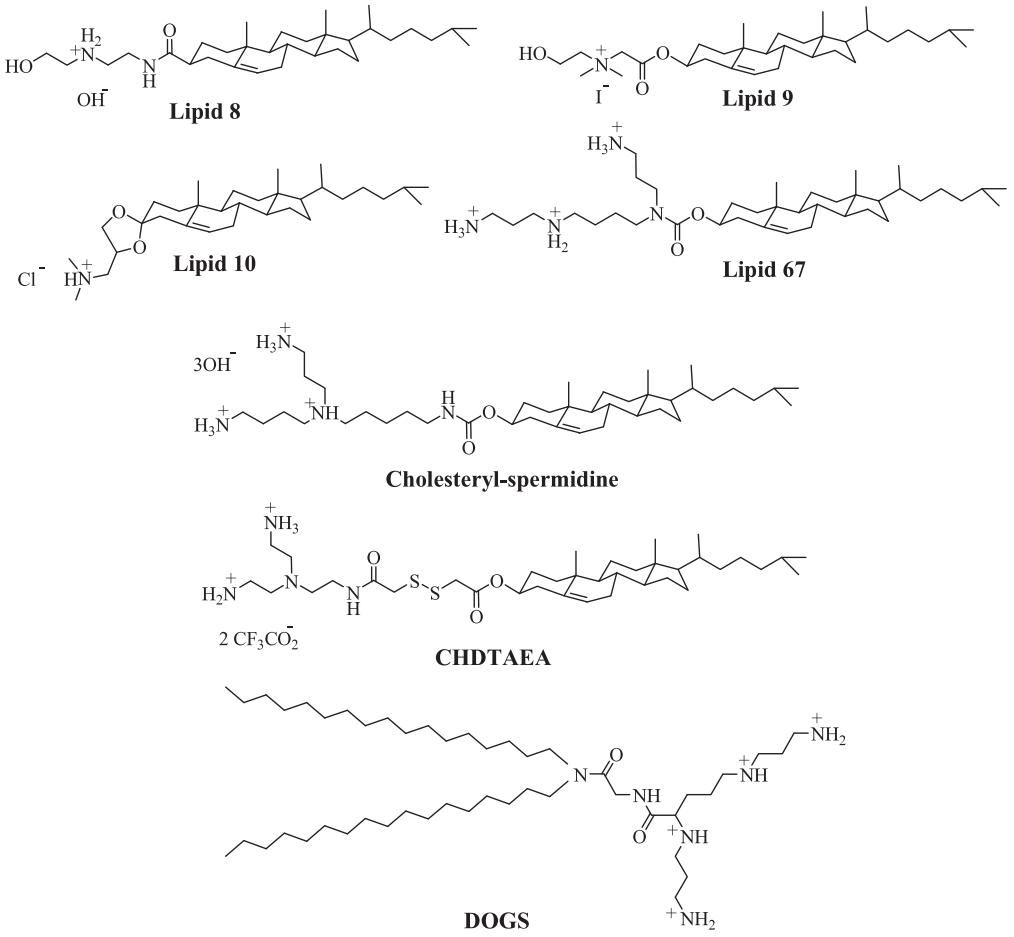
6a: n = 1, R = CH_3
6b: n = 2, R = CH_3
6c: n = 3, R = CH_3
6d: n = 4, R = CH_3
7b: n = 1, R = cis-$(CH_2)_8CH{=}CH(CH_2)_7CH_3$
7d: n = 1, R = CH_2CH_2OH

were incorporated to derive a relationship between the head group hydration and the transfection efficiency. Along with this a flexible fatty acid oleyl chain was appended to **5a**, **5d** and **6a** to study the role of thermally insensitive and rigid hydrophobic fragments. Membranes formed from the aqueous suspensions of these amphiphiles were highly rigid. These aggregates were found to be thermally insensitive,[23] as no solid-to-fluid transition was observed under either temperature-dependent fluorescence depolarization studies or differential scanning calorimetry. Depending on its location in the cholesterol-based monomer, the oxyethylene group showed either hydrophilic or hydrophobic character. In the series **5a–d,** the OEG segment is located outside the hydrophobic portion of the membrane. It takes on hydrophilic character and remains in a random orientation thereby increasing headgroup bulk. Increase in bulkiness of the headgroup increases intermonomer separation, also reflected in the *r* values, which induces interdigitation as supported by XRD data. In the series **6a–d**, the $-(CH_2{-}CH_2{-}O)_n-$ unit is located between the cholesteryl backbone and the $-\mathbf{N^+Me_3}$ headgroup. Here it acts like a hydrophobic spacer as an increase in the *n* value in **6** results in an increase in the length of the bilayer as evidenced from the XRD data.

In the ester-linked series **5a–d**, the transfection efficiency reduced gradually with the progressive introduction of oxyethylene units on a cationic center at a fixed location. In addition, the introduction of oligo-oxyethylene units as a spacer in the linker region of the lipid monomer (Fig. 3) in the ether-linked series **6a–d** also resulted in a marked decrease of the transfection ability. Thus efficient transfection requires a rather "dry" positive charge located as close as possible to the steroid skeleton of the cytofectins. Introduction of a membrane 'disorder'-promoting element like a fatty acyl oleyl chain to **9a** and **6a** resulted in considerable loss of the transfection efficiency. Thus, there seems to exist an optimum balance between the rigidity and the fluidity for this class of cytofectins.

Takeuchi *et al.* reported that replacing the dimethylamino headgroup of DC-Chol (Chart 1) by diethylamino and diisopropylamino groups resulted in a decreased efficiency of gene delivery.[24] This again is in agreement with a relationship between the head group size and the transfection efficiency as the alkyl amino chains may cause steric repulsion between neighbouring head groups. The same author also found that Lipid 8 (cholesteryl-3β-carboxyamido ethylene-*N*-hydroxyethylamine) (Chart 2) was more efficient in gene transfer than its non-hydroxyethylated dimethyl tertiary amino homologue, FRET (fluorescence resonance energy transfer) experiments suggested that Lipid 8 based lipoplexes were particularly unstable in the presence of anionic liposomes.[25] Thus, the transfection ability of Lipid 8 could be related to the lipoplex instability in the endosomes resulting in facilitated DNA release into the cytoplasm. However, the transfection efficiency was dramatically reduced by the addition of a second hydroxyethyl group to the amino group of the lipid. An ether-linked cholesterol conjugate with a dimethyl hydroxyethyl headgroup (cholest-5-en-3β-oxyethane-*N*,*N*-dimethyl-*N*-2-hydroxyethylammonium bromide) was found to be less efficient than its trimethyl non-hydroxyethylated homologue.[21] However, the transfection activity of the ester analogue Lipid 9 (cholesteryl-3β-carboxy-ethylene-*N*,*N*-dimethyl-*N*-2-hydroxyethylammonium iodide) (Chart 2), was found to be related to the lipoplex charge ratio. Indeed, at cation/anion charge ratios up to 5, the hydroxyethylated Lipid 6 was less efficient than its dimethyl tertiary amino and trimethyl quaternary amino homologues. However, its efficiency overtook the methylated homologues at ratios above 7, at

CHART 2.



which the dimethyl and trimethyl homologues were actually inefficient.[21,26]

A rational approach was the incorporation of natural polyamines such as spermidine and spermine, which have a pre-characterized ability to interact with the minor groove of *B*-DNA.[27] Incorporation of the triamine spermidine into Cholesteryl-spermidine (Chart 2),[28] and of the tetraamine spermine into the lipid DOGS (Chart 2),[29] are early representative examples. The presence of protonation sites with different pKa values in Cholesteryl-spermidine and DOGS actually resulted in buffering of the acidic environment of the endosomal compartment, thereby protecting the DNA from degradation and facilitating its escape from the endosome. The multivalent cationic cholesterol derivative Lipid 67 with its T-shaped headgroup (Chart 2) was found to be particularly efficient for gene transfer to the lung and was therefore used in a clinical gene therapy trial for cystic fibrosis.[30–32] Cholesterol has been often used as an alternative to aliphatic chains because of its rigidity, as well as its endogenous biodegradability and fusion activity. Covalently bound cholesterol was first included as the hydrophobic portion of the vector DC-Chol by the Huang group (Chart 1),[3] and then subsequently by others such as BGTC (Chart 1) and its analogues. It is noteworthy that the rigidity of the cholesterol ring may be of interest when lipoplexes with a high degree of physical stability are required for applications such as aerosol delivery. Accordingly, it was found that BGTC/DOPE/DNA lipoplexes resisted to the shear forces involved during nebulisation.[33]

Lipid 10 (cholest-5-en-3-one 3-(dimethylammonium chloride)propylene acetal) (Chart 2) with an acid-sensitive ketal bond, also underwent hydrolysis in acidic medium.[34] Lipid 10 achieved levels of gene delivery comparable to DC-Chol, but the toxicity was correspondingly low. *In vitro* experiments showed a 6 to 15-fold increase in transfection compared to DOTAP, and an up to 50-fold enhanced transfection compared to a non-cleavable analogue.

By employing dithiodiglycolic acid to link the polar and hydrophobic domains, the authors conveyed an increased sensitivity to the disulphide linker. As a result, cleavage of CHDTAEA (cholesteryl hemidithiodiglycolyl tris(aminoethyl)

ammonium trifluoracetate) (Chart 2) could be induced by the endogenous glutathione.[35] It is noteworthy that the increased sensitivity to cleavage rendered the lipid non-cytotoxic.

Interaction of cationic cholesteryl lipids with dipalmitylphosphatidycholine membranes

Effect of natural cholesterol on phosphatidylcholine membranes is well studied.[36–42] We investigated the aggregation properties of functionalized cholesteryl amphiphiles (**5–7**) in water first and then examined their ability to influence the solid gel-to liquid crystalline phase transition of the DPPC bilayer membranes (Fig. 3).[43] We examined the physical changes in membranes comprising mixtures of these cationic cholesterol amphiphiles and DPPC by using transmission electron microscopy (TEM), high sensitive differential scanning calorimetry (DSC), and fluorescence anisotropy using a membrane soluble probe 1,6-Diphenyl-1,3,5-hexatriene (DPH).

TEM images showed that aggregates of DPPC alone formed long, open lamellar type of structures. With increasing amount of cholesteryl lipid such as **5a** or **5b** to an extent of 30 mol-% in DPPC membranes, closed membranous aggregates with high curvature were observed. But even after inclusion of 30 mol-% of **6c** (which has a long hydrophobic $-CH_2-$ chain) in DPPC, closed aggregates were not seen.

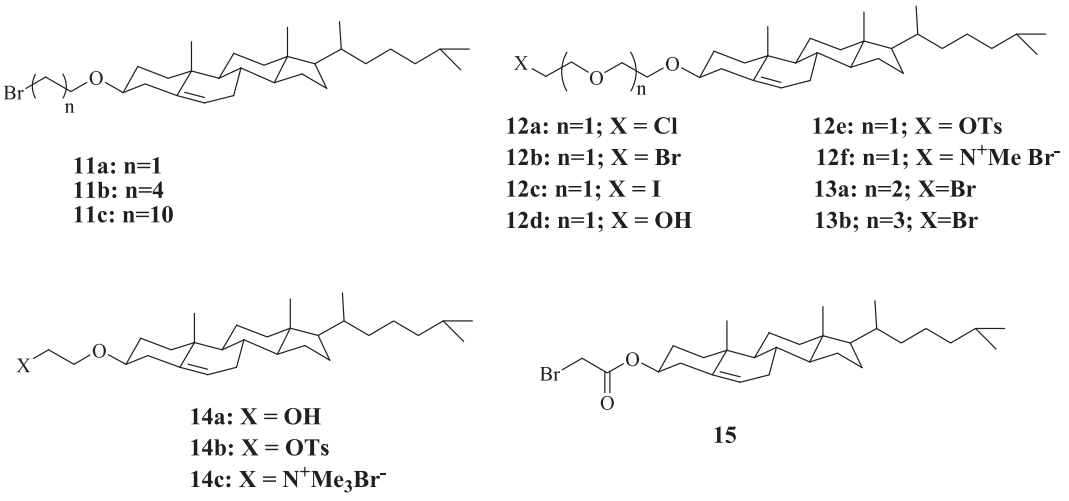
Membranes based on fatty acid linkedphosphatidylcholine DPPC manifested a sharp inflection at $\sim 42^{\circ}C$ indicating the solid gel-to-liquid crystalline phase transition. On the other hand, for neat aqueous suspensions of cholesteryl lipids **5–7**, irrespective of their headgroup structures, only gradual monotonic decreases of r-values with increase in temperature were observed after which a plateau was reached. Thus, no typical melting temperature (T_m) profile.

Though these molecules possess a charged polar segment attached to cholesterol, the resultant cationic cholesteryl lipids are able to quench the chain motions of the acyl chains of DPPC, thus functioning as filler molecules like cholesterol. However, some of them induced perturbations into the DPPC membrane in a significantly different manner than cholesterol. In general, the ester-linked lipids **5a–c** have greater tendency towards rigidification of the fluid phase than others did. Also, these lipids upon incorporation in increasing concentrations not only abolished the phase transition and depressed the temperature at which the transition occurred but also induced a curvature of membranes of DPPC. This suggests that the ester-linked cholesteryl lipids **5a–c** associate themselves with PC molecules differently than the ether-based amphiphiles **6a–c**. This was probably due to the presence of dipole-induced dipole interactions that held the ester and carbonyl oxygen atoms of the DPPC and cholesteryl lipid monomers close to each other.

Cholesterol derived non-hydrolysable systems as single component LCs

Compounds with cholesteric mesophases are being used as circular polarizers, band pass filters, wide band reflective polarizers etc. most of them show the cholesteric (liquid crystals) LC phase only at high temperatures.[44,45] To circumvent this, mixtures of selected compounds have been used and shown to form LCs at room temperature.[46] Thus scientists always look for compounds which have cholesteric mesophase at ambient temperature. Many derivatives of cholesterol have been shown to possess liquid-crystalline properties.[47–49] Most of them are cholesterol derivatives based on ester, carbonate or urethane linkages, which are unfortunately susceptible to hydrolysis in moisture which limits their applications. We have synthesized a number of ether linked ω-haloalkyl derivatives of cholesterol which show excellent liquid crystalline (LC) properties at room temperature.[50] Since these are based on non-hydrolysable ether linkages, they possess long term stability. A bromine atom was attached via polymethylene spacer ($n = 1, 4, 10$) and oligo-oxyethylene spacer to cholesteryl backbone in series **11a–c** and **12b**, **13a–b** respectively. In **13a–f**, different terminal groups are present while in **15**, there is an ester linkage. Among all the compounds, **11a–c** and **12a–c** showed liquid crystalline property. While **16a-c** showed a cholesteric mesophase with platelet like texture, compounds **12a–c** showed cholesteric mesophase with a planar texture. DSC thermograms of phase transitions in **11a–c** and **11a–c** showed that LC property of these molecules depend on the type and length of the spacer between the bromine atom and the steroid backbone. In cholesterol based liquid-crystals, imbalance between a flexible moiety and rigid cholesteryl skeleton results in the formation of a mesophase. Stable mesophases were formed with compounds having short to medium spacer lengths. Polyoxyethylene spacers, being long, confer too much flexibility which destabilizes their mesophase as in **13a** and **13b**. Thus, there is a delicate balance between the mobility of the spacer chain and the rigid cholesterol backbone to which it is attached. Even excess rigidity disfavours the formation of mesophase as is seen with **15** where the spacer is linked to cholesterol backbone via an ester rather than an ether type of linkage. The conformational requirements of the

Figure 4: Molecular structures of cholesteric amphiphiles with different linkers and head groups.



ester carbonyl group and possible dipole-induced dipole interactions between the carbonyl center and ester oxygen of adjacent molecules probably impose greater rigidity on spacer due to which the liquid-crystalline property is lost.

Role of halide counter ion in liquid crystalline properties

In order to gain an insight into the effect of end group in inducing liquid crystalline property, bromo moiety was replaced with: (1) –OH which could interact via hydrogen bond (**12d**); (2) a flat aromatic group like a tosylate (**12e**); (3) a charged unit with bromine species as a counter ion like $\mathbf{NMe_3^+Br^-}$ (Fig. 4). In all these cases liquid crystalline property was lost. However, when the bromo moiety was replaced with a chloro or an iodo moiety, as in **12a** or **12c** respectively, the liquid-crystalline property was still retained. Thus the halogen moiety is crucial for this property. Interestingly, the nature of the halogen atom does not seem to induce significant variations in the liquid-crystalline textures although it affects the thermal properties.

Selective reflection of polarized light

It is well known that cholesteric LCs reflect circularly polarized light at selected wavelengths at a given temperature depending on the pitch of the helix formed in the mesophase. If the angle between the cholesteric helix and incident light is changed, depending on the angle of observation, different colours of reflection are observed. Now, when the temperature is increased, the pitch of the helix also increases which manifests in the selective reflection of the polarized light at a different wavelength. Mesophases of **12a–c** oriented well and showed a sharp absorbance of light, which changed with temperature. At 46°C, **12a** showed green color which changed to green at 34°C, then changed to lemon yellow at 26.5°C and red at 17.5°C. **12b** and **12c** also behaved similarly. Though the λ_{max} of **12a–c** decreased with increase in T, the range of λ_{max} spanned by them in their mesophase regimes are different. Thus we were successful in synthesizing a number of ether-linked ω-haloethoxy-ethanol derivatives of cholesterol which represented the first examples of room temperature, single component, cholesteric LC class of molecules (Fig. 4). They displayed iridescent colors spanning entire visible region. Temperature-dependent UV study of the wavelength of maximum reflection showed that the color of these films could be modulated to reflect selected wavelengths.

Conclusions

Cholesterol-based cationic lipids can be conveniently prepared and characterized, and thereby compared in order to generate the structure-activity relationships which are required for achieving design of a second generation systems from initial design are to take place. As the biological barriers and chemical environments that the lipoplexes traffic through are still far from understood, a wide variety of cholesterol-based cationic lipids have been designed *via* more or less empirical modifications to headgroup, hydrophobic domain and linker. Enhanced gene delivery remains a potential for such systems. Similarly it has been possible to derive a predictable structure-activity relationship from a single component cholesterol based systems showing

room temperature liquid-crystalline properties. An important attribute to this class of molecules is their non-hydrolysable character and long term stability.

Acknowledgement

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Abbreviations

BGTC	= Bis-guanidinium-tren-cholesterol
CHDTAEA	= Cholesteryl hemidithiodiglycolyl tris(aminoethyl)ammonium trifluoracetate
DC-Chol	= 3β-[N-(N', N'-dimethylaminoethyl) carbamoyl) cholesterol)
DEAE-Dextran	= Diethylaminoethyl-Dextran
DOGS	= Dioctadecylamido-glycylspermine
DOPE	= Dioleoyl phosphatidylethanolamine
DOTAP	= 1,2-dioleoyloxy-3-[trimethylammonio]-propane
DOTMA	= N-(1-(2,3-dioleyloxy)propyl)-N, N, N-trimethylammonium chloride)
DNA	= Deoxyribonucleic acid
DPH	= 1,6-Diphenyl-1,3,5-hexatriene
DPPC	= L-α-Dipalmitoyl phosphatidylcholine
DSC	= Differential scanning calorimetry
FRET	= Fluorescence Resonance Energy Transfer
LCs	= Liquid Crystals
PC	= Phosphatidylcholine
PEG	= Polyethylene glycol
T_m	= Melting Temperature
TC-Chol	= 3β-[N-($N\prime$,$N\prime$,$N\prime$-trimethylaminoethane) carbamoyl] cholesterol
TEM	= Transmission electron microscopy

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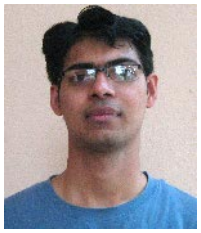

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