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A Brief Study on Liposomes-A Review

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Abstract

Liposomes are a novel drug delivery system (NDDS), in which the medication is encapsulated in a vesicle. It has been a study interest in the development of a NDDS. Liposomes are colloidal spheres of cholesterol non-toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins and drug molecules or it is also called vesicular system. It is differ in size, composition and charge. It is a drug carrier loaded with great variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids. Few drugs are also formulated as liposomes to improve their therapeutic index. Consequently a number of vesicular drug delivery systems such as liposomes, niosomes, transfersomes, and pharmacosomes were developed. The focus of this review is to the various method of preparation, characterization of liposomes, advantages and brings out the application vesicular systems.

Keywords: NDDS, Liposomes, Vesicles, Colloidal spheres

Contents

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1. Introduction

Dispersion of phospholipids in water, which spontaneously form a closed structure with internal aqueous environment bounded by phospholipids bilayer membranes, this vesicular system is called as liposome [1]. Liposomes are the small vesicle of spherical shape that can be produced from cholesterols, non-toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins [2]. Liposomes are the drug carrier loaded with great variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids. Liposomes were discovered about 40 years ago by A.D. Bangham [3], which has become the versatile tool in biology, biochemistry and medicine today. In 1960s, liposome has been used as a carrier to transport a wide variety of compounds in its aqueous compartment. Liposome can be formulated and processed to differ in size, composition, charge and lamellarity. To date liposomal formulations of anti-tumor drugs and antifungal agents have been commercialized [4]. The clinical potential of liposomes as a vehicle for replacement therapy in genetic deficiencies of lysosomal enzymes was first established in 1970s [5, 6]. Considerable progress was made during 1970s and 1980s in the field of liposome stability leading to long circulation times of liposomes after intravenous administration resulting in the improvement in bio-distribution of liposome. The important anti-tumour drug doxorubicin had been formulated as liposome in 1980s to improve the therapeutic index. There are several mechanisms by which liposomes act within and outside the body which are as follows [7]:

1. Liposome attaches to cellular membrane and appears to fuse with them, releasing their content into the cell.
2. Sometimes they are taken up by the cell and their phospholipids are incorporated into the cell membrane by which the drug trapped inside is released.
3. In the case of phagocyte cell, the liposomes are taken up, the phospholipid walls are acted upon by organelles called lysosomes and the active pharmaceutical ingredients are released.

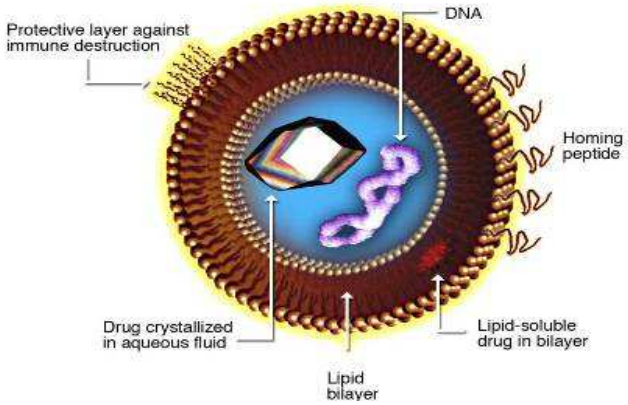

Figure1. Structure of Liposome taken from google.com

Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shell(s) consisting of lipids arranged in a bilayer configuration. The potential use of liposomes as drug carriers was recognized more than 25 years ago [8] and, since that time, liposomes have been used in a broad range of pharmaceutical applications (Table 1). This review first highlights some of the key advances of the past decade in the design of liposomes for systemic delivery and then reviews the most recent literature involving specific applications of liposomal drug-delivery systems. Liposome technology Preparation of liposomes Liposome can be prepared by a variety of methods (extensively reviewed in [9, 10]). In general, on the basis of size and lamellarity (number of bilayers present within a liposome), liposomes are classified into three categories: multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), and small unilamellar vesicles (SUVs). Drug loading can be achieved either passively (i.e. the drug is encapsulated during liposome formation) or actively (i.e. after liposome formation). Hydrophobic drugs, such as amphotericin B. taxol or annamycin, can be directly incorporated into liposomes during vesicle formation, and the extent of uptake and retention is governed by drug-lipid interactions. Trapping efficiencies of 100% are often achievable, but this is dependent on the solubility of the drug in the liposome membrane. Passive encapsulation of water-soluble drugs relies on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation. Trapping efficiencies (generally <30%) are limited by the trapped volume contained in the liposomes and drug solubility. Alternatively, water-soluble drugs that have protonizable amine functions can be actively entrapped by employing pH gradients [11], which can result in trapping efficiencies approaching 100% liposomes with prolonged circulation lifetimes A significant advance in the development of liposomal drugs has come with the use of specialized lipids, such as monosialoganglioside GM, or polyethylene glycol modified phosphatidyl ethanolamine (PEG-PE), that engender long circulation lifetimes when incorporated into liposomes [12-14].

Alternatively, the presence of entrapped cytotoxic drug can also lead to extended circulation times [15]. It has been demonstrated that increased circulation lifetimes enhance the opportunity for liposomes, administered systemically, to leave the vascular compartment and enter certain extravascular regions [16-18] Tumors, for example, exhibit leaky blood vessels that have a reduced ability to retain circulating macromolecules [19,20]. Liposomes can extra vasate in these regions, thus leading to preferential accumulation within tumors. Studies have now clearly demonstrated that long-circulating liposomes containing PEG-PE or cytotoxic drugs, such as doxorubicin, accumulate within these sites preferentially compared with conventional liposomes [16,18, 21]. Targeted delivery It is envisioned that the next generation of liposomal pharmaceuticals will consist of drug-loaded liposomes with surface-associated targeting information (Pig. I). Site-directing targeting ligands, such as monoclonal antibodies, can be attached to liposomes by either covalent or non-covalent methods [22-24]. The advent of novel PEG-PE lipids that allow targeting ligands to 700 Pharmaceutical biotechnology be conjugated at the distal ends of the PEG spacer has afforded both effective target binding in vitro and prolonged circulation times.[25-28]. To date, only two studies have demonstrated the improved therapeutic activity of liposomal drugs in vivo achieved through the use of antibody-mediated targeting [29,30], with both studies employing a monoclonal antibody against lung endothelial thrombomodulin (mAb 34A) and intravenously injected tumor cells. The use of immunoliposomes may be limited because of their potential immunogenicity [31]. In addition to antibodies, glycolipids (e.g. galactose [32] and

mannose [33]), proteins (e.g. transferrin [34] and asialofetuin [35]), and vitamins (e.g. folic acid [25', 36]) have been used to target specific cells via cell surface receptors. Intracellular delivery Liposomes can facilitate the intracellular delivery of drugs by fusing with the target cell. Alterations in the lipid composition can render liposomes pH sensitive, leading to enhance fusogenic tendencies in low Ph compartments such as endosomes [37]. The inclusion of lipids that is able to form non-bilayer phases, such as dioleoylphosphatidyl ethanolamine (DOPE), can promote destabilization of the bilayer, inducing fusion events. DOPE has been particularly useful for cationic liposomes complexed with plasmid DNA for gene delivery [38,39].

2. Liposome Preparation Method [40-77]

A) Multilamellar Liposomes (MLV)

(i) Lipid Hydration Method

(a) This is the most widely used method for the preparation of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities. MLV are simple to prepare by this method and a variety of substances can be encapsulated in these liposomes. The drawbacks of the method are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous (Bangham et al., 1965, 1974).

(b) MLVs with high encapsulation efficiency can be prepared by hydrating the lipids in the presence of an immiscible organic solvent (petroleum ether, diethyl ether). The contents are emulsified by vigorous vortexing or sonication. The organic solvent is removed by passing a stream of nitrogen gas over the mixture. MLVs are formed immediately in the aqueous phase after the removal of organic solvent (Papahadjopoulos and Watkins, 1978; Gruner et al., 1985). The main drawback of this method is the exposure of the materials to be encapsulated to organic solvent and to sonication.

(ii) Solvent Spherule Method

A method for the preparation of MLVs of homogeneous size distribution was proposed by Kim et al. (1985). The process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath.

B) Small Unilamellar Liposomes (SUV)

(i) Sanitation Method

Here MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere. The main drawbacks of this method are very low internal volume/encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV along with SUV. Recently, Oezden and Hasirci (1991) prepared polymer-coated liposomes by this method.

(ii) French Pressure Cell Method

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials (Hamilton and Guo, 1984). The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 ml maximum).

(iii) A new method for the preparation of SUV was given by Lasic et al. (1987). They deposited egg phosphatidylcholinc mixed with 1.5 %w/v of cetyl tetramethylammonium bromide (a detergent) in CHCI3/CH3OH on various supports for example silica gel powder, zeolite X, zeolite ZSM5. After the removal of organic phase, the system was resuspended by shaking or stirring in distilled water or 5 mM NaCl. There was some loss of phospholipid (about 10-20%) due to adsorption on the supports. The loss was 70% and 95% in the case of silica gel and zeolite ZSMS respectively.

A homogenous population of vesicle with average diameter of 21.5 nm was obtained when zeolite X (particle size of 0.4 mm) was used as a support.

C) Large Unilamellar Liposomes (LUV)

They have high internal volume/encapsulation efficiency and are now days being used for the encapsulation of drugs and macromolecules.

(i) Solvent Injection Methods

(a) Ether Infusion Method

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is

heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature (Dcamcr and Bangham, 1976; Schieren et al., 1978).

(b) Ethanol Injection Method

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol (Batzri and Korn, 1973).

(ii) Detergent Removal Methods

The detergents at their critical micelles concentrations have been used to solubilize lipids. As the detergent is removed the micelles become progressively richer in phospholipid and finally combine to form LUVs. The detergents were removed by dialysis (Kagawa and Rocker, 1971; Milsmann et al., 1978; Alpes et al., 1986). The advantages of detergent dialysis method are excellent reproducibility and production of liposome populations, which are homogenous in size. The main drawback of the method is the retention of traces of detergent(s) within the liposomes. A commercial device called LIPOPREP (Diachema AG, Switzerland), which is a version of dialysis system, is available for the removal of detergents. Other techniques have been used for the removal of detergents:

(a) by using Gel Chromatography involving a column of Sephadex G-25 (Enoch and Suitt matter, 1979),

(b) by adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-2 (Gerristen et al., 1978).

(c) by binding of octyl glucoside (a detergent) to Amberlite XAD-2 beads (Philippot et al., 1985).

(iii) Reserves Phase Evaporation Method

First water in oil emulsion is formed by brief sonication of a two-phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands (Szoka and Papahadjopoulos, 1978). We get a heterogeneous sized dispersion of vesicles by this method. Modified Reverse Phase Evaporation Method was presented by Handa et al. (1987) and the main advantage of the method is that the liposomes had high encapsulation efficiency (about 80%). The Reverse Phase Evaporation Method of Szoka and Papahadjopoulos (1978) have also been modified to entrap plasmids without damaging DNA strands (Haga and Yogi, 1989).

(iv) Calcium-Induced Fusion Method

This method is used to prepare LUV from acidic phospholipids. The procedure is based on the observation that calcium addition to SUV induces fusion and results in the formation of multilamellar structures in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs (Papahadjopoulos and Vail, 1978). The main advantage of this method is that macromolecules can he encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, although of a heterogeneous size range. The chief disadvantage of this method is that LUVs can only be obtained from acidic phospholipids.

(v) Microfluldization Method

Mayhew et al. (1984) suggested a technique of microfluidization/microemulsification/ homogenization for the large-scale manufacture of liposomes. The reduction in the size range can be achieved by recycling of the sample. The process is reproducible and yields liposomes with good aqueous phase encapsulation. Riaz and Weiner (1995) prepared liposomes consisting of egg yolk, cholesterol and brain phosphatidylserin diasodium salt (57:33:10) by this method. First MLV were prepared by these were passed through a Microluidizer (Microlluidics Corporation, Newton, MA, USA) at 40 psi inlet air pressure. The size range was 150-160 nm after 25 recylces. In the Microluidizer, the interaction of fluid streams takes place at high velocities (pressures) in a precisely defined micro channel, which are present in an interaction chamber. In the chamber pressure reaches up to 10,000 psi this can be cause partial degradation of lipids.

(vi) Extrusion under nitrogen through polycarboriate filters LUV can be prepared by passing MLV under nitrogen through polycarbonate membrane filters (Jousma et al., 1987). The vesicles produced by this method have narrow size distribution. The extrusion is done under moderate pressures (100-250 psi). A special filter holder is required. Such devices are available commercially under the trade names such as LUVET and EXTRUDER and are equipped with a recirculation mechanism that permits multiple extrusions with little difficulty. Small quantities of liposome preparations (about 10 mL) can be easily prepared by the help of a commercial extruder. Riaz and Weiner (1994) prepared liposomes by this technique. The liposomes contained phosphatidylcholine from egg yolk and crude phosphoinositide sodium salt in the ratio of 4:1 and the lipid concentration was 12.5 /mole/ml. MLVs were passed through Extruder Lipex Membrane Inc., Vancouver, Canada) ten times through a stalk of two 100 nm polycarbonate filters (Nudeopore Pleasanton, CA, USA) employing nitrogen pressures upto 250 psi. Freeze fracture electron

microscopy and p31-FT NMR revealed that the liposomes were unilamellar. Photon Correlation Spectroscopy revealed that the size range was 99-135 nm.

(vii) Lasic et al. (1988) reported a method for the instant formation of a rather homogeneous preparation of LUV by a simple technique. The formation of multilammelar liposomes is prevented by inducing a surface charge (+ ve) on the bilayer while the size of the vesicles is controlled by the topography of the wafer support surface on which phospholipid film was formed. They deposited 0.5-1.0 mg egg yolk lecithin doped with 3 ml of CHCl3/CH3OH on a specially etched 2-inch silicon wafer. This wafer was put in place of the original bottom of an Erlenmeyer flask that is bottom of the flask is replaced by wafer. After having dried overnight at 102 tore (about 1 Pa), the film was resuspended by gentle shaking in 1-2 ml water. Liposomes were formed instantly. The contamination of liposomes with large structures such as MLVs, giant vesicles and phospholipids particles was ruled out by video enhanced phase contrast microscopy.

(viii) A method for the extemporaneous preparation of LUVs has been described by Liautard and Phillippot (1993). The method was recommended for immediate clinical use of liposomes.

(ix) Freeze-Thaw Method

SUVs are rapidly frozen and followed by slow thawing. The brief sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is due to the fusion of SUV during the processes of freezing and or thawing (Pick, 1981; Ohsawa et al., 1985; Liu and Yonethani, 1994). This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipid concentration. The encapsulation efficiencies from 20 to 30% were obtained (Pick, 1981).

(D) Giant Liposomes

(i) The procedure for the formation of giant liposomes involves the dialysis, of a methanol solution of phosphatidylcholine in the presence of methyl glucoside detergent against an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm (Oku et al., 1982).

(ii) A method for the formation of giant single lamellar liposomes with size in the range of 10 to 20 prn by the removal of sodium trichloroacetate by dialysis was presented by Oku and MacDonald (1983).

(E) Multivesicular Liposomes

(i) The formation of multivesicular liposomes has been reported by Kim et al. (1983). The water in oil emulsion was converted to organic solvent spherules by the addition of the emulsion to across solution. The evaporation of organic solvent resulted in the formation of multivesicular vesicles. The diameter of liposomes ranges from 5.6 to 29 pm. The materials which can be encapsulated include glucose, EDTA, human DNA. These liposomes have very high encapsulation efficiency (up to 89%).

(ii) Cullis et al. (1987) found that when MLV preparations were subjected to five cycles of freeze on liquid nitrogen)-thaw and followed by thawing in warm water, the liposomes of high encapsulation efficiency (up to 88%) could be obtained. Freeze fracture electron micrographs revealed vesicles within vesicles.

(F) Assymetric Liposomes

It has been shown that the phospholipid distribution in natural membranes is asymmetric. For example phosphatidytcholine and sphingomyelin concentrate at the outer half of lipid bilayer whereas phosphatidyl ethanolamine, phosphatidyl inositoland phosphatidylserine are mainly localized in the inner half of bilayer (Op den Kamp, 1979). Due to this, attempts have been made to prepare LUVs in which phospholipid distribution in both halves of bilayer is different. It appears that as model membranes the asymmetric liposomes are nearer to natural membranes than the conventional unilamellar liposomes. In the latter the phospholipids distribution is symmetrical in bilayer.

(i) Cestaro et al. (1982) described a procedure for the preparation of asymmetric liposomes which contain cerebroside sulfate only at the outer leaflet of phospholipids bilayer. Cerebroside sulfate was adsorbed on to a filter paper (cellulose) support and then the support was incubated with small or large fused unilamellar liposomes. After six hours sulfatide contents reached about 6 mole percentage of the total quantity of phospholipid, corresponding to about 10 mole % of phospholipid present in the outer layer. The sulfatide could not be removed by washing with 1M NaCl or 1M urea.

(ii) Pagano et al. (1981) reported the formation of asymmetric phospholipid vesicles, which contained fluorescent lipid analogue in either the outer or inner leaflet of the liposome bilayer. The procedure is based on the observation that the lipid analogues undergo rapid exchange (transfer) between the vesicles populations.

(iii) Denkins and Schroit (1986) prepared asymmetric liposomes by the enzymatic conversion of the fluorescent lipid-analogue of phosphatidylserine (NBD-PS) in the outer leaflet of LUV to NBD- phosphatidylethanolamine (NBD-PE).

(iv) Low and Zilversmit (1980) reported that lipid exchange proteins could be effectively being used so remove phosphatidylinositol at the outer leaflet of unilamellar liposomes. Therefore, it appears that these proteins may be used for the preparation of asymmetric liposomes.

(v) Collis et al. (1987) found that in SUV, distribution of lipid was not symmetrical and ratio of lipid in the outer monolayer to lipid in the inner monolayer could be as large as 2:1. Therefore, small unilamellar liposomes can be also be called as asymmetric to some extent.

3. Application of Liposome

Cationic liposomes for gene delivery:

Among various synthetic carriers currently in use in gene therapy, cationic liposomes are the most suitable transfecting vectors. Gene encapsulation in liposomal vesicles allows condensation of DNA plasmid into a highly organized structure, and protects DNA against degradation during storage and in the systemic circulation of the gene encoding a therapeutic protein. Moreover, structural organization of the gene-delivery system must bypass the cell membrane and facilitate endosomal escape, avoiding DNA degradation in the lysosomal compartment (Figure 2).

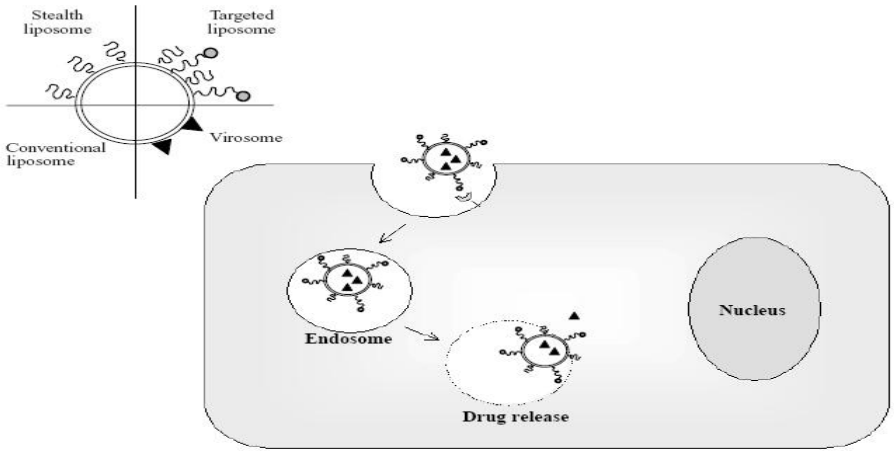

Figure 2: Schematic representation of conventional, stealth, targeted liposomes, and virosomes. Among different mechanism of intracellular uptake of liposomes, endocytosis of targeted liposomes is exemplified.

Numerous cationic lipids have been tested in the formulation of liposomes for gene delivery (the structural formulas of some of them are presented in Figure 3). Transfection efficiency is strongly affected by the presence of three components in the structure of these lipids: a positively charged head-group that interacts with negatively charged DNA, a linker group (which determines the lipid's chemical stability and biodegradability), and a hydrophobic region to anchor the cationic lipid into the bilayer. Among these, the most often used are N-[1-dioleyloxy)propyl]-N,N,N-trimethylammonium (DOTMA) and dioleoylphophatidylethanolamine (DOPE) in a 1:1 phospholipid mixture (Lipofectin®, Invitrogen Corporation, Carlsbad, CA, USA). Other commercially-available lipids are 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanammonium trifluoroacetate 1,2-bis(oleoyloxy)-3-(trim ethylammonio)propane (DOTAP), 1,2-dimystyloxypropyl-3-dimethylhydroxyethyl ammonium bromide (DMRE), 3β[N-(N',N'-dimethylaminoethane)-carbomoyl] cholesterol (DC-CHOL), and dioctadecylamino-glycyl-spermine (DOGS or Transfectam®)[78].

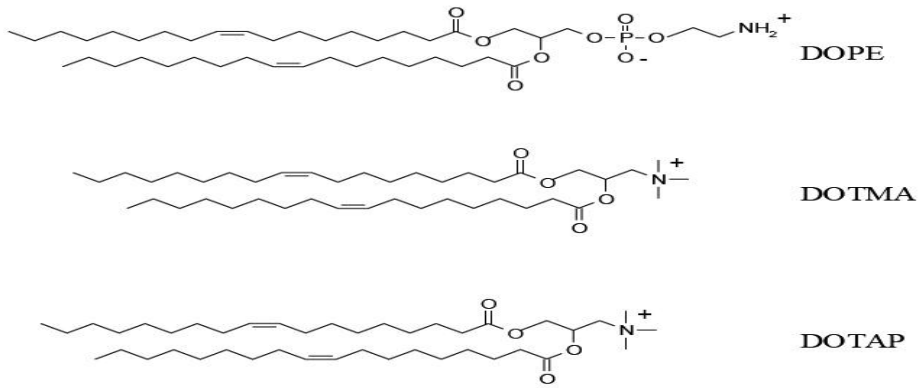

Figure 3: Chemical structures of the cationic lipids: dioleoylphophatidylethanolamine (DOPE), N-[1- dioleyloxy) propyl]-N, N, N-trimethylammonium (DOTMA) and 1, 2-bis (oleoyloxy)-3-(trimethylammonio) propane (DOTAP).

Nevertheless, the clinical use of cationic liposomes is limited by their instability, rapid clearance, large particle size, toxicity at repeated administration, and induction of immuno stimulation and complement activation. Water-soluble lipopolymers obtained by conjugating different fatty acid chains to branched polethylenimine (PEI) of 25 kDa or above, have been shown to be effective for gene delivery; they can be delivered into the cytoplasm after endosomal

disruption. Similarly, phosphatidyl ethylene glycol (PhosEG) has been linked to the amino group of branched PEI [78]. On the other hand, PEGylation of cationic liposomal vesicles is a promising alternative way to overcome these problems, prolonging circulation time in vivo and increasing accumulation at the disease site, even if the transfecting efficiency might be significantly reduced In liposomes composed of a cationic lipid (DOTAP, DOGS, dimethyldioctadecylammonium bromide-DDAB), a neutral lipid (DOPE) and a phospolipid derivative of PEG (PEG-PE), complexing 18-mer phosphothioate as a model for active oligodexyribonucleotide (ODN), surface modification with a relatively large amount of PEG (5.7 mol%) has been showed to improve ODN loading without losing structural activity or stability of the resulting complexes, retaining size without vesicle aggregation. Moreover, the hydrophilic shell of PEG enhances the in vitro stability by evading mononuclear phagocyte clearance, and retains a high level of the originally loaded ODN in the complex after plasma incubation. Only after modification of PEG cationic liposomes with targeting agents can cytoplasmatic delivery of DNA material be observed. The PEG-modified complex conjugated anti-HER2 F (ab') dramatically enhanced cell uptake, increasing diffuse cytoplasmatic and nuclear localization of ODN in SK-BR-3 cells [79].

In liposome composed of DODAC/DOPE, the inclusion of 5 mol% of PEG lipid conjugate did not inhibit uptake by the cell membrane of lipid/DNA complex, but substantially modified the ability of the cationic liposomal carrier to disrupt the endosomal membrane. Endosomal escape into the cytoplasm depended on the acyl chain of the lipid complex and on the molecular weight of the PEG. Optimizing the desorbtion rate of PEG-lipids may be one approach to overcoming the inhibitory effect on intracellular delivery of plasmid [80]. To contrast the low tranfection efficiency of PEG-modified cationic liposomes due to the absence of a net positive charge on the vesicle surface, a series of cationic PEG-lipids with one or more positive charges have been synthesized and designed for post-insertion in preformed stabilized plasmid-lipid particles (Figure 4). Incorporation of cationic-poly (ethylene glycol)-lipid conjugates (CPL4) in DOPE/DODAC/PEG-CerC20 liposomes resulted in both improved uptake into BHK cells and dramatically enhanced transfection potency in the presence of Ca^{2+}, which assists in destabilizing the endosomal membrane following uptake. However, in this type of liposomal preparation, aggregation of vesicles was observed, probably due to formation of H-bonding between the amino and carbonyl groups present in the distal head-group at the end of the PEG chain [81]. In order to optimize CLP-liposomes for systemic delivery, the length of PEG linker in the CPL can be modulated. When the PEG3400 linker extended beyond the PEG-CerC20 "cloud" was employed for liposomal insertion, charged liposomal systems were produced that rapidly cleared from circulation; it was suggested that a shorter PEG linker might be used, such as PEG1000, allowing the PEG-CerC20 to shield the positive charge of CPL. Moreover, PEG-CerC20 can be designed to slowly dissociate at the disease site, achieving exposure of the CPL at the target area with retention of long-circulation properties and interaction between liposomes and targeting cell [82].

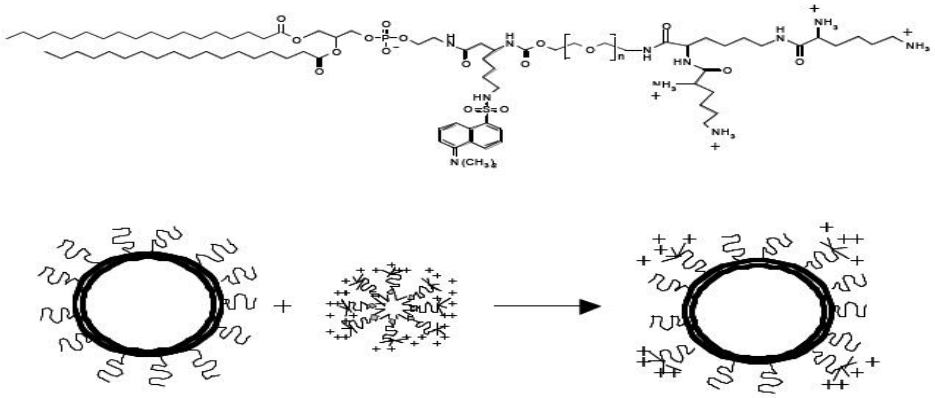

Figure 4: Structure of dansylated cationic-poly (ethylene glycol)-lipid (CPL_4) and schematic representation of the post-insertion method for the production of CPL_4 liposomes (redrawn from Palmer et al 2003).

Overall, the most suitable use of PEG is as a tether for a specific ligand on the surface of these systems, in order to obtain a target-specific gene delivery facilitating internalization in cells and endosomal escape. Cell-penetrating peptides (CPP), such as Trans-activating transcriptional activator (TAT), homeodomain of antennapedia (Antp), herpes simplex virus type I protein VP22 and transportan, have been reported to guarantee direct cytosolic delivery when coupled with several carriers, including liposomes. Multiple TATp molecules can be attached on the surface of liposomes via the spacer group of p-nitrophenylcarbonyl-PEG-phosphatidylethanolamine. TATp-liposomes-DNA complexes were found to be capable of transfection of both normal and cancer cells in vitro and in vivo with lower cytotoxicity that the commonly used lipid-based gene delivery systems [83].

Liposomes for diagnostic imaging:

Actively or passively targeted liposomes can be used as carriers for contrast agents to increase the signal difference between areas of interest and background, and to specifically localize the contrast moieties in the target tissues or organs. The versatility of liposomal vesicles to carry different types of compound in the bilayer or in the aqueous compartment makes them suitable for all contrast procedures, including gamma-scintigraphy, magnetic resonance imaging (MRI), computed tomography imaging (CTI), and sonography. Using liposomes in diagnostic imaging leads to several advantages, owing to their capability to incorporate multiple contrast moieties, to specifically deliver the agent to the target area, and to enhance the contrasting signal. In order to incorporate diagnostic agents (^{111}In, ^{99}Tc, Mn, Gd, etc) in liposomes, metals can be complexed with a soluble chelating agent (such as DTPA) that will be encapsulated in the aqueous core of the vesicles. Alternatively, the chelating compound complexing with the metal can be derivatized with a hydrophobic group for insertion in the lipid bilayer. Gd-DTPA complexes were the first to be incorporated in the aqueous core. Among the various lipophilic DTPA–conjugates that have been synthesized, DTPA-sterylamine (DTPA-SE) and DTPA-phosphatidyl ethanolamine (DTPA-PE) show reduced leakage and toxicity of potentially toxic metals. (DTPA-polylysil) glutaryl phosphatidyl ethanolamine (DTPA-PLL-NGPE) is a poly-chelating amphiphilic polymer suitable for liposome incorporation that drastically increases the number of metal ions attached to a single lipid conjugate. In these cases, metals are situated on the liposomal surface, directly exposed to the aqueous environment, thus enhancing the contrast proprieties. To increase the stability and half-life of vesicles in the body after administration, liposomes for use as contrast agent can be modified with PEG. DTPA-PLL-NGPE liposomes with PEG5000 containing Gd improved visualization in the lymph nodes: PEG moieties increase the amount of water directly in contact with the Gd on the liposomal surface, and contrast phagocytic cell uptake at the injection site.

Long-circulating Gd liposomes have been successfully used for blood pool imaging, prolonging the presence of the contrast agent in the body. After systemic administration of Gd-DTPA-PLL-NGPE/PEG-liposomes, the signal was immediately clear and lasted for up to 4 hours [84].Incorporation of large amount of Gd-containing lipids in sterically stabilized PEGylated DSPC- or DOPC-based liposomes showed increased relaxivity compared with traditional Gd-DTPA; because of the higher accessibility of water, liposomes containing unsaturated phospholipids also showed increased relaxivity in comparison with liposomes composed of saturated phospholipids. These liposomes are therefore highly potent contrast agents for application in MR imaging [85].Liquid-filled liposomes have been demonstrated to be echogenic. The liquid-like composition of the vesicles makes them more resistant to pressure and mechanical stress than encapsulated gas microbubbles. Moreover, their long circulation characteristics and their small size are favorable in echography. Definity® (Bristol-Myers Squibb Medical Imaging, Inc. New York, NY, USA) is a contrast agent containing perfluoropropane with a phospholipid shell approved in the US for use in cardiology. After lyophilization, liposomes can encapsulate small amounts of air, being echogenic upon rehydration. Is it possible to modulate the liposomal composition by changing the ratio between PC, PE, PG, and CHOL to produce agents that are echogenic in vitro and in vivo [86]. Echogenic liposomes have also been utilized for intravascular ultrasound imaging; targeting the vesicles to the vascular signature associated with arteroma development [87].A pH liposomal MRI contrast agent has recently been introduced as a potential marker of low pH in tumor interstitium. DPPE/DSPG/GdDTPA-BMA liposomes displayed increased relaxivity in the blood when the pH was below the physiological level, due to aggregation and leakage of GdDTPA-BMA. To optimize these liposomal formulations it is necessary that they retain pH sensitivity in the blood and accumulate in the tumor. Blood circulation time was prolonged by incorporating 1.5mol% in DPPE/ DSPG liposomal GdDTPA-BMA, but the pH-response was reduced. A compromise would be necessary between long blood retention and pH-sensitivity [88].

Liposomes for vaccines

Genetic vaccination-encoding antigens from bacteria, virus, and cancer have shown promise in protecting humoral and cellular immunity. The success of liposomes-based vaccines has been demonstrated in clinical trials and further human trials are also in progress. Liposomes are of interest as carriers of antigens, especially because they act as effective adjuvants for the immune system response, without causing granulomas at the injection site and producing no hypersensitivity reactions [89]. Liposome formulations would also protect their DNA content from deoxyribonuclease attack. Moreover, their transfection efficiency could be improved by modulating surface charge, size, and lipid composition of the vesicle and entrapping additional adjuvant or immunostimulator compounds in the antigen formulation. Several strategies have been followed to target liposomes to cell receptors, such as antibodies (or Fc-γ) or branched chain mannose moieties. Cationic or pH-sensitive liposomes that are able to release their contents into the cytoplasm following endocytosis have also been developed. Two commercial vaccines based on virosome technology are currently on the market. Epaxal® (Berna Biotech Ltd, Bern, Switzerland), a hepatitis A vaccine, has inactivated hepatitis A virus particles adsorbed on the surface of the immunopotentiating reconstituted influenza virosomes (IRIV). In Inflexal® V (Berna Biotech Ltd) the virosome components themselves are the vaccine protective antigens [90]. Virosomes are liposomal formulations that have viral envelope proteins anchored to their lipid membrane. The lipid bilayer is composed of PC intercalated with the virus-derived proteins

hemagglutinin and neuraminidase. These virus-like particles have proven to be effective immunogens with unique adjuvant properties [91]. Liposome-encapsulated malaria vaccine contains monophosphoryl lipid A as adjuvant in the bilayer and the formulation is adsorbed on aluminum hydroxide. In a Phase I dose-escalating study, the formulation showed induction of higher level of anti-malaria antibody in human volunteers [89]. Some liposome formulations are under investigation in preclinical studies against Yersina pestis, ricin toxin and Ebola Zaire virus. Liposomes against ricin or pestis, composed of PC/CHOL/DDA containing KWC vaccine, were administered intranasally to C57BL/6 mice; liposome formulations gave higher protection from infection than KWC in buffer. Liposomes composed of PC and CHOL containing ricin toxoid and ricin A-chain (rA) increased antibody responses to the rA chain. Liposomes composed of DMPC/DMPG/CHOL with or without lipid A containing Ebola Zaire virus have been tested in mice and cynomolgus monkeys [92]. Liposome vaccination also has the potential to be a powerful weapon in cancer treatment. Chen et al developed a novel liposome-based system for the delivery of plasmid DNA. Lipid-polycation-DNA particles are formed by combining cationic liposomes and polycation-condensed DNA organized in a virus-like structure able to release its content in the cytoplasm. Cationic liposomes promote a much higher humoral and cytotoxic T lymphocyte immune response against the antigen encoded by the entrapped DNA vaccine. Liposome-stabilized prostate cancer vaccine is under investigation in a series of Phase I trials in patient with advanced prostate cancer. The new liposome-lipid A-prostate-specific antigen formulation showed greater safety and higher immunological potency than other formulations and has been transitioned to Phase II trials [89].

4. Conclusion

In the development of novel drug delivery system (NDDS) liposomes has the various applications, which are utilized as a carrier for therapeutic molecules. Liposomes are highly useful for cancer therapy and vaccination. DOXIL, SPI077, Lipoplatin, S-CKD-602 have been approved or in advance trial of PEGylated liposomal formulations. PEG-derivatized liposomes with increased stability can easily be modified using a wide array of targeting moieties (MAb, ligands) to deliver the drug specifically to the target tissues with increasing accuracy. The development of liposome delivery to particular subcellular compartments is a field of great interest in different fields, such as gene therapy and vaccination. The interaction of stealth liposomes with cell membranes, and release of the drug in the neighborhood of target tissues are still under investigation, but some recent studies indicate that the use of detachable PEG may facilitate cell penetration and/or intracellular delivery of vesicles. PEG-coated liposomes are becoming increasingly important, giving technological and biological stability to liposomal systems.

5. References

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