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Liposome as a Carrier for Advanced Drug Delivery

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Abstract: Liposomes are being a potential carrier for advanced drug delivery. These are usually spherical lipid bilayers ranging from 50 to 1000 nm in diameter and serve as a convenient delivery vehicle for therapeutically active drug (small molecule, macromolecules like protein/peptide/Gene). Phospholipid aggregates are easy to form and vulnerable to structural manipulations, allowing for the adjustment of their properties for particular purposes. In selected cases, the application of liposomes in pharmacological therapy improves drug pharmacokinetics compared to its free form. The major advantages of the liposome application are the protection of active compounds from degradation; the increase in circulation time and the possibility to achieve partial or total selectivity. Selectivity alone improves drug potency, eliminates side effects and allows for dosage reduction. In this project the liposomes were reviewed for its nature, type, composition, preparation, mechanism of drug-transport, strategy of targeting and potential applications in respect with the advanced drug delivery system.

Key words: Liposomes, drug delivery, cancer therapy, encapsulation of drug, gene transfer, vaccination

INTRODUCTION

The field of drug delivery is advancing rapidly (Langer, 2001). By controlling the precise level and/or location of drug in the body, side effects are reduced, lower doses are often needed and new therapies are possible (Langer, 1998).

Liposomes have been receiving a lot of interest as a carrier for advanced drug delivery (Bangham, 1983). Liposomes were first produced in England in 1961 by Alec D. Bangham, who was studying phospholipids and blood clotting. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule is water soluble, while the opposite end is water insoluble. Water-soluble medications added to the water were trapped inside the aggregation of the hydrophobic ends; fat-soluble medications were incorporated into the phospholipid layer.

A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drugs or genetic material into a cell. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine), or of pure components like DOPE (dioleolylphosphatidylethanolamine).

The lipid bilayer can fuse with other bilayers (e.g., the cell membrane), thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs, (which would normally be unable to diffuse through the membrane), they can be (indiscriminately) delivered past the lipid bilayer. The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection. Liposomes can be created by sonicating phospholipids in water. Low shear rates create multilamellar liposomes, which have many layers like an onion. Continued high-shear sonication tends to form smaller unilamellar liposomes.

In this review work we have investigated the recent domains of liposome based drug delivery as well as a future exploration of this system in DDS.

TYPES OF LIPOSOMES

Depending upon the structure there are two types of lipsomes (Thomas and Joseph, 2001; Gregoriadis, 1993): *Unilamellar* liposomes: Unilamellar vesicle has a single phospholipid bilayer sphere enclosing aqueous solution (buffer).

Multilamellar liposomes: Multilamellar vesicles have onion structure. Typically, several unilamellar vesicles will form one inside the other in diminishing size, creating a multilamellar structure of concentric phosphlipid spheres separated by layers of water (Fig. 1).

Conventional liposomes:
Stabilized natural lecithin (PC) mixtures
Synthetic identical-chain phospholipids
Glycolipid containing liposomes

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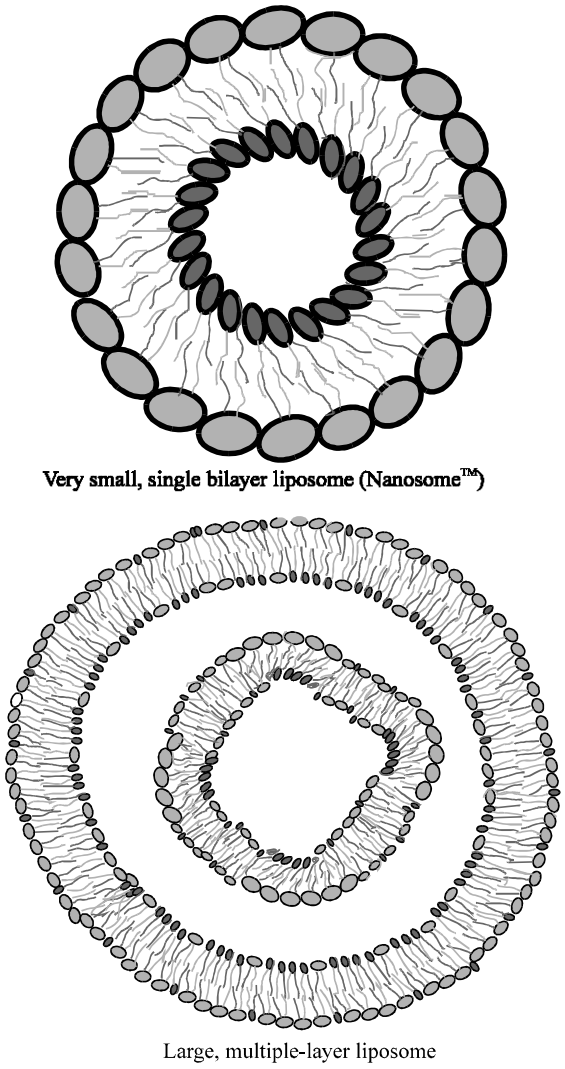

Fig. 1: Single bilayer and Multiple layer liposomes

Specialized liposomes:

- Bipolar fatty acids
- Antibody directed
- Methyl/methylene X-linked
- Lipoprotein coated
- Carbohydrate coated
- Multiple encapsulated
- Emulsion compatible

There are another special type of liposomes getting popularity now a days namely Stealth liposome and liposome with homing device.

Stealth liposome: For conventional liposomes removal from the circulation is too fast to benefit from this escape mechanism (Woodle *et al*., 1992). Thus, long circulation

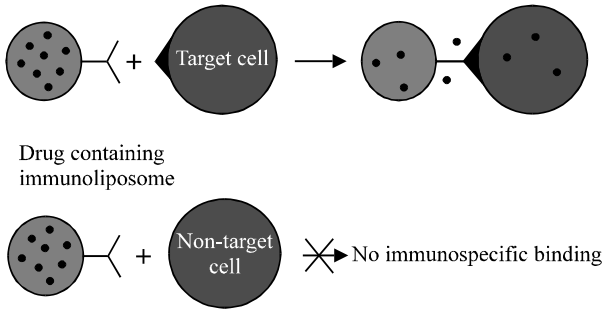

Fig. 2: Principle of drug targeting with immunoliposome

times of liposomes were required to take full advantage of this 'leaky endothelium' effect. This brings us to the second important finding. Coating liposomes with polyethylene glycol (PEG) reduces the rate of uptake by macrophages ('stealth' effect) and leads to a prolonged presence of liposomes in the circulation and consequently provides ample time for these liposomes to escape from the circulation through leaky endothelium.

This 'stealth' principle has been used to develop the successful doxorubicin-loaded liposome product that is presently marketed as Doxil or Caelyx for treatment of solid tumours. Recently, impressive therapeutic improvements were described by using corticosteroid-loaded liposomes in experimental arthritic models.

By far the focal point regarding the application of long circulating liposomes has been on their potential to escape from the blood circulation. However, these long circulating liposomes may also act as a reservoir for prolonged release of a therapeutic agent. Woodle *et al*., (1992) reported on a remarkably long pharmacological action of vasopressin when formulated in long circulating liposomes.

Liposomes with 'homing' devices: How can one make liposome uptake tissue- or cell-specific? Figure 2 shows liposomes with antibodies covalently attached to the surface of the liposomes. Indeed, most work on liposome targeting has been done with antibodies or antibody fragments attached to the surface (Mastrattista *et al*., 1999). But other homing devices have been considered as well.

For example, plasminogen-coated liposomes were designed to specifically reach fibrin clots (plasminogen has an affinity for fibrin) to deliver fibrinolytics. More recently, reports appeared on RGD peptide-driven targeting of liposomes to endothelial cells to block angiogenesis. Also, saccharide-directed targeting has been described, for example, the use of saccharide-antennae (including galactose) to direct liposomes to hepatocytes.

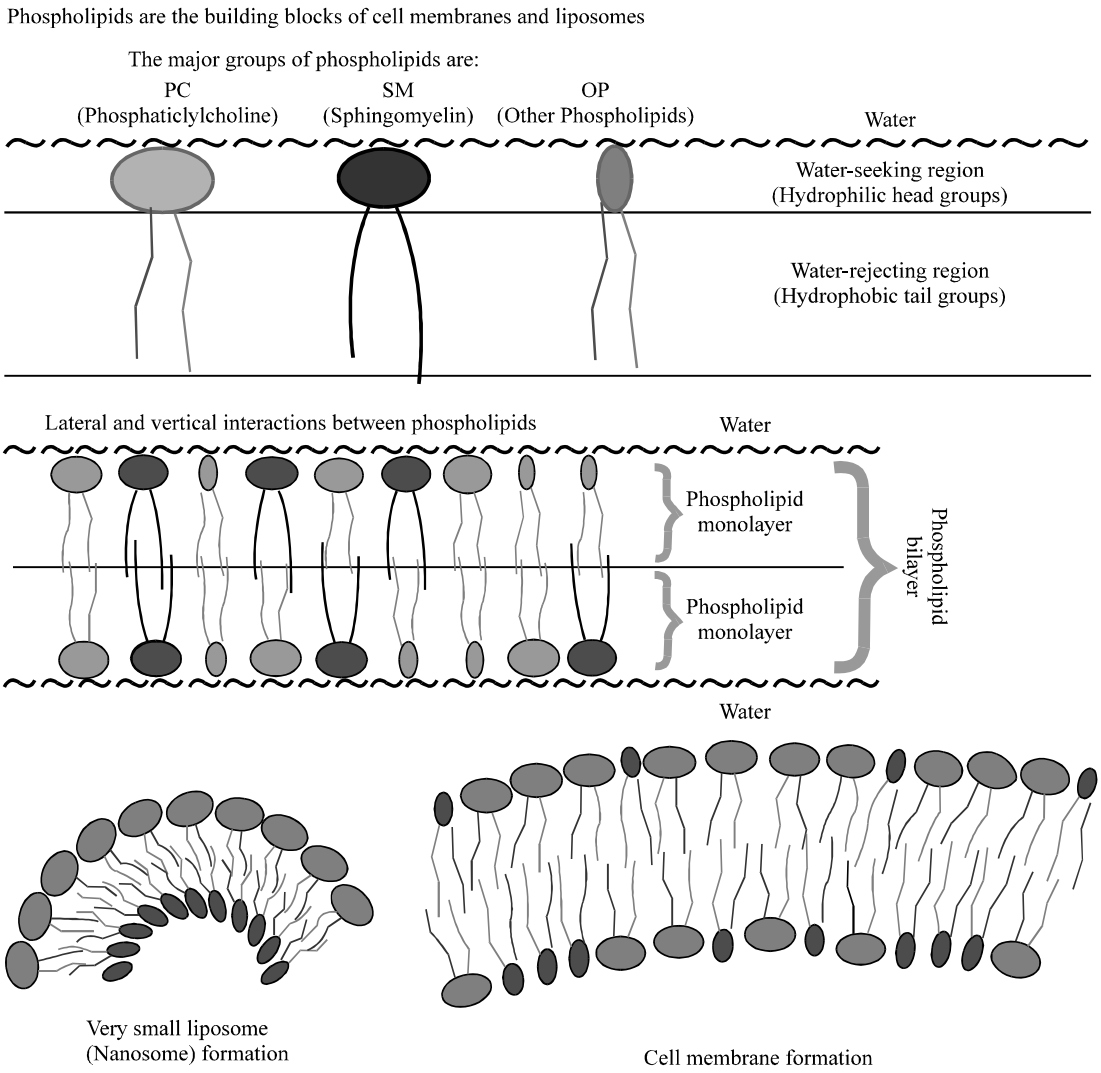

Fig. 3: Compositional structure of liposome and also a cell membrane

Targeted liposomes should have immediate access to the target site and should not be taken up by macrophages before encountering their target tissue or cells. Therefore, nowadays 'stealth' technology is often combined with attachment of a homing device to the terminal end of the PEG chain that is exposed to the aqueous medium. Many examples of successful targeting in the blood compartment have been described.

Compositions and characteristics of liposomes: Usually liposomes composed of cholesterol and phospholipids (such as phosphatidylcholine and dicetylphosphate)-the structure, composition and proportion being practically the same as in the host cell membranes (Manconi *et al*., 2002). The phospholipids possess a hydrophobic tail structure and a hydrophilic head component and organize in the following manner when dissolved in water: the hydrophobic tails mutually attract, while the hydrophilic heads contact with the aqueous medium external and internal to the liposome surface (Fig. 3). In this way, double lipid layers are formed which seal off to form small vesicles similar to the body cells and their organelles. These spheres or liposomes constitute small deposits that can be made to contain an antigen, an antibiotic, an allergen, a drug substance or a gene (as in gene therapy). The liposomes can in turn be introduced in the body without triggering immune rejection reactions. Phospholipid Bilayers are the core structure of liposome and cell membrane formations.

Mechanism of transportation through liposome: Liposome can interact with cells by four different mechanisms (Thomas and Joseph, 2001).

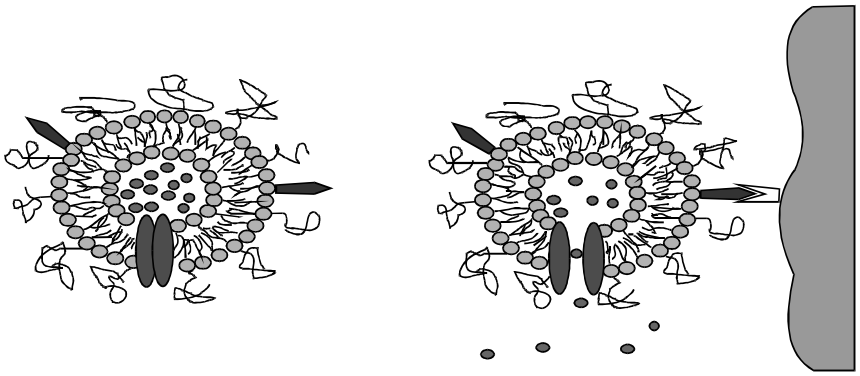

Fig. 4: Mechanism of transportation through liposome

- Endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils.
- Adsorption to the cell surface either by nonspecific weak hydrophobic or electrostatic forces or by specific interactions with cell-surface components.
- Fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal content into the cytoplasm.
- Transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents (Fig. 4).

It often is difficult to determine what mechanism is operative and more than one may operate at the same time.

FORMULATION FACTOR OF LIPOSOME

In formulating with liposomes, procedures and raw materials must be considered carefully to avoid adverse effects on liposome stability (Kadir *et al.*,1999).

- In general, liposomes should be added to a formulation below 40°C using low shear mixing.
- The addition of liposomes should also be the last step in the formulation's manufacturing process.
- Ethyl alcohol concentration should be kept below 5%, solvents should be kept below 10%.
- Surfactants in general should also be avoided, but low levels (up to 1%) of non-ionic high HLB surfactants are usually well tolerated.
- High levels of salts (>0.5%) should be avoided.
- The recommended storage temperature of most liposome formulations is 25°C.

Table 1: Solubility characteristics in liposome preparation

Solvent	Solubility
Deionized Water	Dispersible suspension
100% ethanol	Insoluble
25:75 alcohol:water	Dispersible suspension*
50:50 alcohol:water	Dispersible suspension*
75:25 alcohol:water	Insoluble
Corn oil	Insoluble
Olive oil	Insoluble
Mineral oil	Insoluble
Butylene glycol	Insoluble
Propylene glycol	Partially soluble (breakup of liposomes)
Silicone oil	Insoluble
Isononyl isononanoate	Insoluble
SLES surfactant	Dispersible suspension**

*Ethyl alcohol concentration should be kept below 5%, solvents should be kept below 10%, **Surfactants in general should also be avoided, but low levels (up to 1%) of non-ionic high HLB surfactants are usually well tolerated

Solubility characteristics in liposome preparation: The solubility characteristics were furnished according to the solvent (Table 1) as in every case solute-solvent behaves just 'like solvent like solute'.

LIPOSOME PREPARATION

By dialysis or gel filtration methods Preparation of Liposomes in various methods (Crommelin *et al.*, 1999) such as:

Hand-shaken method: In order to produce liposomes lipid molecules must be introduced into an aqueous environment. When dry lipid film is hydrated the lamellae swell and grow into myelin figures. Only mechanical agitation provided by vortexing, shaking, swirling or pipetting causes myelin figures (thin lipid tubules) to break and reseal the exposed hydrophobic edges resulting in the formation of liposomes. Large multilamellar liposomes can be made by hand-shaken method.

Sonication method: This method is probably the most widely used method for the preparation of small unilamellar vesicles. There are two sonication techniques:

Probe sonication: The tip of a sonicator is directly immersed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The dissipation of energy at the tip results in local overheating and therefore the vessel must be immersed into an ice/water bath. During the sonication up to one hour more than 5% of the lipids can be de-esterify. Also, with the probe sonicator, titanium will slough off and contaminate the solution.

Bath sonication: The liposome dispersion in a tube is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method compare to sonication the dispersion directly using the tip. Material being sonicated can be kept in a sterile container, unlike the probe units, or under an inert atmosphere.

The lipid bilayer of the liposome can fuse with other bilayers (e.g., cell membrane), thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be delivered past the lipid bilayer.

Reverse-phase evaporation method: Historically this method provided a breakthrough in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid ratio and able to entrap a large percentage of the aqueous material presented. Reverse-phase evaporation is based on the formation of inverted micelles. These inverted micelles are formed upon sonication. These inverted micelles are formed upon sonication of a mixture of a buffered aqueous phase, which contains the water soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. The slow removal of the organic solvent leads to transformation of these inverted micelles into a gel-like and viscous state. At a critical point in this procedure, the gel state collapses and some of the inverted micelles disintegrate. The excess of phospholipids in the environment contributes to the formation of a complete bilayer around the remaining micelles, which results in formation of liposomes. Liposomes made by reverse phase evaporation method can be made from various lipid formulations and have aqueous volume-to-lipid ratios that are four times higher than multilamellar liposomes or hand-shaken liposomes.

Freeze-dried rehydration method: Freeze-dried liposomes are formed from preformed liposomes. Very high encapsulation efficiencies even for macromolecules can be achieved using freeze-dried hydration method. During the dehydration the lipid bilayers and the materials to be encapsulated into the liposomes are brought into close contact. Upon reswelling the chances for encapsulation of the adhered molecules are much higher. The rehydration is a very important step and in should be done very carefully. The aqueous phase should be added in very small portions with a micropipette to the dried materials. After each addition the tube should be vortexed thoroughly. As a general rule, the total volume used for rehydration must be smaller than the starting volume of the liposome dispersion.

Detergent depletion method: The detergent depletion method is used for preparation of a variety of liposomes and proteoliposome formulations. Detergents can be depleted from a mixed detergent-lipid micelles by various techniques which leads to the formation of very homogeneous liposomes. In practice all lipids below their phase transition temperature can be used with this preparation method. Not all detergents are suited for this method and only a few detergents can be used for detergent depletion method. The most popular detergent are sodium cholate, alkyl (thio) glucoside and alkyloxypolyethylenes. Mixed micelles are prepared by adding the concentrated detergent solution to multilamellar liposomes (the final concentration of the detergent should be well above the critical micelle concentration (CMC) of the detergent). Equilibrium of the mixed micelles in the aqueous phase takes quite some time and the equilibrium does not happen during a short period of time. The use of different detergents results in different size distributions of the vesicles formed. Faster depletion rates produces smaller size liposomes. The use of different detergents also results in different ratios of large unilamellar vesicles/oligolamellar vesicles/multilamellar vesicles.

Detergent depletion is achieved by of four following approaches

Dialysis: The dialysis can be preformed in dialysis bags immersed in large detergent free buffers (equilibrium dialysis) or by using continuous flow cells, diafiltration and cross filtration.

Gel filtration: In this method the detergent is depleted by size exclusive chromatography. Sephadex G-50, Sephadex G-100, Sepharose 2B-6B and Sephacryl S200-S1000 can be used for gel filtration. The liposomes do not penetrate into

the pores of the beads packed in a column. They percolate through the inter-bead spaces. At slow flow rates the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids, therefore pre-treatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

Absorption: Detergent absorption is achieved by shaking of mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads and Bio-beads SM2. The great advantage of the using detergent adsorbers is that they can remove detergents with a very low critical micelle concentration (CMC) which are not completely depleted.

Dilution: Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer the micellar size and the polydispersity increases dramatically and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from polydisperse micelles to monodisperse vesicles occurs.

HOW TO PRESERVE LIPOSOME

Once lipid particles have been formed, maintaining the physical properties of the particles can be difficult. Size distribution can change on storage due to degradation of the components. Permeabilization of the membrane can lead to leakage of encapsulated material (Aso and Yoshioka, 2005). Stability issues due to hydrolytic degradation are a general problem with lipid products. Aqueous formulations of drug products tend to be less stable since the presence of excess or bulk water leads to rapid hydrolytic degradation in lipid preparations.

After the sizing process is complete, lipid suspensions should be stored at close to pH 7 as possible. Lipids containing ester-linked hydrocarbon chains are susceptible to acid and base hydrolysis. Hydrolysis rate is dramatically affected by temperature therefore lipid suspensions should be kept refrigerated during storage. Lipid suspensions should not be frozen as the freezing process could fracture or rupture the vesicles leading to a change in size distribution and loss of internal contents. The use of cryoprotectants such as dextrose, sucrose and trehalose may increase stability from hydrolysis. Also, samples may experience oxidation upon storage. The addition of small amounts of antioxidants during processing may stabilize the suspension and limit oxidation of the product. SUV should be stored above their transition temperature for no longer than ~24 h. LUV may be store for a longer period of time if stored at 4-8°C when not in use. Hydrolysis of the lipid begins to occur immediately resulting in monoacyl derivatives (Lyso lipids) which act as detergents and disrupt the membrane, thus permeabilizing the membrane. After ~5-7 days at 4-8°C the internal contents will begin to leak indicating hydrolytic degradation of the lipid. If membrane structure is not a critical parameter in your experiments, vesicles may be stored for 1-2 months with minimal (<10%) hydrolytic degradation.

In general, liposomes suspensions should not be frozen as the freezing process could fracture or rupture the vesicles leading to a change in size distribution and loss of internal contents. Depending on the application for the liposomes, changes in particle size can have a dramatic effect on functionality.

Storage time depends on a number of factors including temperature, pH, medium, etc. Liposomes stored in a buffer at pH 7.4 and at ~4°C did not display membrane structural changes for 5-7 days as demonstrated by retention of a trapped fluorescent marker. Beyond that time the fluorescent marker began to leak out of the liposome indicating the presence of membrane destabilizing components, presumably lyso lipid and free fatty acid generated by hydrolysis of the lipid.

PROSPECTS OF LIPOSOME FOR DRUG TARGETING STRATEGIES

How can liposome targeting lead to tissue-specific therapeutic effects? Upon interaction with the target cell, a number of approaches have been proposed and some of those have proven to give therapeutic advantages in animal models. Below we briefly discuss the more successful approaches so far (Mastrattista *et al.*, 1999).

Strategy I: The immunoliposomes are interacting with a cell surface receptor that is endocytosed, leading to immunoliposome internalisation upon immunoliposome-cell interaction. For a successful action of the liposome-associated drug, escape from the endosome is often required as many drugs are inactivated when the endosome matures from the endosomal state into a lysosome.

For endosomal escape, fusogenic peptides (often derived from viruses such as influenza) have been proposed or, alternatively, pH-dependent liposomes are

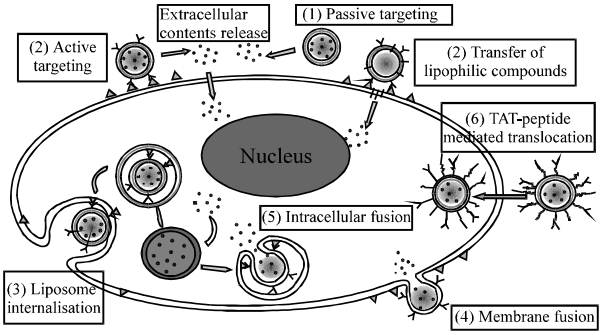

Fig. 5: Potential ways by which targeted immonoliposomes can achieve cytolic drug delivery: passive (1) and active (2) targeting, receptor mediated endocytosis (3), fusion with the plasma membrane (4) or intracellulary (5) or TAT-mediated translocation

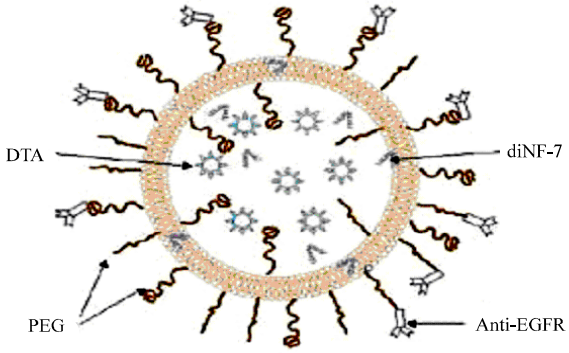

Fig. 6: Peptide-inducedrelease of 'stealth liposome'-entrapped diphteria toxin (DTA), a liposome-dependent drug. The peptide diNF-7 is a fusogen based on the N-terminal domain of influenza virus HA-2 and is activated upon a pH drop; anti-EGFR acts as 'homing' device

used that destabilise the endosomal membrane when the pH level drops (Fig. 5). The liposome structure that is now required is built of several components with their specific functions: the liposome as carrier; the antibody as homing device; PEG as stealth-coat; the fusogen as endosomal escape tool and (last but not least) the drug. This drug preferably belongs to the category 'liposome-dependent drugs' because then maximal advantage can be obtained from the drug targeting approach. An example of a liposome-dependent drug is the a chain of diphtheria toxin. Without a carrier that delivers this compound into the cytosol it is inactive. Only in the cytosol it exerts its extremely high toxicity by blocking ribosome activity very efficiently (Fig. 6).

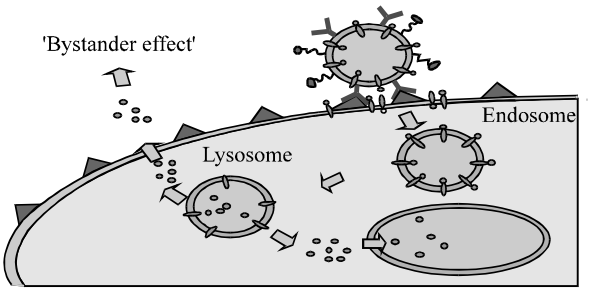

Fig. 7: Schematic presentation of a selective transfer model proposed for a lipophilic prodrug of the anti-cancer agent FudR-dP from immunoliposomes to the plasma membrane of tumour cells. After target cell binding, the immunoliposome incorporated FUdR-dP is transferred to the plasma membrane of the tumour(1). The prodrug is internalized (2) and hydrolysed intralysosomally (3). The active drug FUdR then diffuses into the cytoplasm (4) from where it is either transferred into the nucleus (5, site of action) or extracellulary (6, bystander effect)

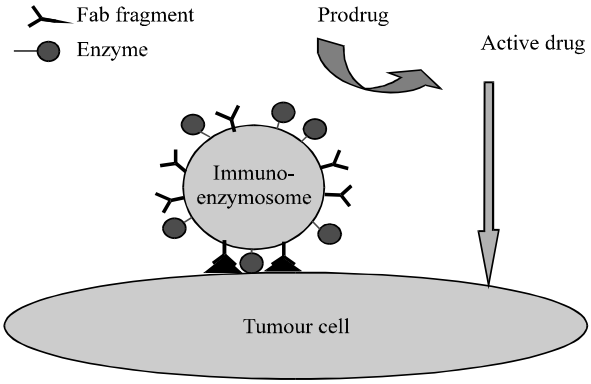

Fig. 8: Concept of antibody -directed enzyme prodrug therapy (ADEPT) with immunoliposomes. The immuno-enzymosomes are first allowed to bind to target cells. Then a prodrug is given, which is activated by the immuno-enzymosomes in close proximity to the target cell. Subsequently, the active drug can kill the cell

Strategy II: An elegant strategy developed by Egberts (2002) is based on the selective binding of immunoliposomes that contain a lipophilic prodrug. This prodrug is selectively transferred from the cell-bound immunoliposomes into the target cell. Subsequently the prodrug is converted in the lysosome into the active drug. From the lysosome it leaks into the

cytoplasm and possibly even to the outside of the cell causing a so-called 'bystander effect' (Fig. 7).

Strategy III: In antibody-dependent enzyme prodrug therapy (ADEPT), a prodrug is converted only at sites where its converting enzyme is delivered. Site-specific delivery of the enzyme is performed by using a site-specific antibody. To make target site enzyme delivery more efficient, one can attach enzymes to immunoliposomes. Now many enzyme molecules can be delivered to the target site on one targeted immunoliposome (Fig. 8).

Considering the complexity of these three targeting strategies and the strong desire in the pharmaceutical world 'to keep things simple', well-established technologies such as liposome 'stealth' technologies and other existing drug carrying lipid complexes are preferred in the short-term.

Only in those cases where these 'simple' solutions fail and new strategies for life threatening diseases are on the drawing board will the industry further develop those strategies that have been successful in an academic setting. Every component of such complex structures as depicted in Fig. 6 should be carefully chosen to fit into the delivery strategy and challenges will be encountered regarding reproducibility, stability and up-scaling methodologies.

ADVANCED APPLICATION OF LIPOSOMES

Liposomes are used to deliver certain vaccines, enzymes, or drugs (e.g., insulin and some cancer drugs) to the body. When used in the delivery of certain cancer drugs, liposomes help to shield healthy cells from the drugs' toxicity and prevent their concentration in vulnerable tissues (e.g., the kidneys and liver), lessening or eliminating the common side effects of nausea, fatigue and hair loss. Liposomes are especially effective in treating diseases that affect the phagocytes of the immune system because they tend to accumulate in the phagocytes, which recognize them as foreign invaders. They have also been used experimentally to carry normal genes into a cell in order to replace defective, disease-causing genes. Liposomes are sometimes used in cosmetics because of their moisturizing qualities.

Liposome in vaccination: In terms of vaccine development (Kunisawa *et al*., 2001; Alving, 1990), cytoplasmic delivery is a crucial for the induction of the cytotoxic T lymphocyte (CTL) responses that play a pivotal role against infectious diseases and cancer. In this context, it was reported that fusogenic liposomes could deliver encapsulated antigens into the cytoplasm and induce MHC (Major Histocompatability Complex) class I-restricted, antigen-specific CTL responses. In addition, fusogenic liposomes are also effective as a mucosal vaccine carrier

Liposomes are avidly phagocytosed by macrophages and other cells of the reticuloendothelial system. As a result, they make excellent adjuvants for many purified antigens. An example that can be used to explain this principle is represented by a bacterial exopolysaccharide or a recombinant protein. These elements are expensive to produce and purify, though when inoculated into liposomes in small amounts, an adequate immune response can be achieved.

Liposome in gene transfer: Direct gene transfer for the treatment of human diseases requires a vector which can be administered efficiently, safely and repeatedly (Gao and Huang, 1993). Cationic liposomes represent one of the few examples that can meet these requirements. Currently, more than a dozen cationic liposome formulations have been reported. These liposomes bind and condense DNA spontaneously to form complexes with high affinity to cell membranes. Endocytosis of the complexes followed by disruption of the endosomal membrane appears to be the major mechanism of gene delivery. The effectiveness and safety of this DNA delivery method has been established in many studies. Based on these results, two human gene therapy clinical trials using cationic liposomes have been conducted and more trials will be started in the near future. The simplicity, efficiency and safety features have rendered the cationic liposome an attractive vehicle for human gene therapy.

A gene transfer vector has been developed utilising anionic liposomes as a carrier of plasmid DNA (pEGlacZ, 7.6 kb) to transfect $CD3^+$ T lymphocytes (Jurkat cells) (Turner *et al*., 2002a). The plasmid DNA that contained the *Escherichia coli* β-galactosidase reporter gene was condensed using poly-l-lysine of molecular mass 20,700 (PLK_{99}) to form a polyplex which was interacted with several anionic liposome formulations to form lipopolyplexes. The liposome formulations where based on dioleoylphosphatidylethanolamine (DOPE) in combination with cholesterol and dioleoylphosphatidylcholine (DOPC) and oleic acid, or dimyristoylphosphatidylethanolamine (DMPE). For targeting to the Jurkat cells distearoylphosphatidylethanolamine (DSPE) linked to poly (ethylene glycol) molecular mass 2000 and coupled to anti-CD3 antibody was incorporated. The polyplexes

and lipopolyplexes were characterised in terms of size, zeta potential, agarose gel electrophoresis and electron microscopy and the permeability of the lipopolyplexes to liposome-encapsulated glucose was determined (Turner *et al*., 2002b). The polyplexes consisted of a mixed population of rod-like structures (53-160 nm long and 23-31 nm diameter) and spheres (18-30 nm diameter). The lipopolyplexes retained a permeability barrier although were more permeable to glucose than their component liposomes. The poly-l-lysine condensing agent was still susceptible to pronase digestion suggesting that the polyplex was associated with the outer surface of the liposome. The lipopolyplexes with lipid composition DOPE/cholesterol/OA/DSPE-PEG2000 anti-$CD3^+$ PLK_{99}-plasmid DNA had significant gene transfer activity, as monitored by β-galactosidase expression, that depended on the charge ratio of the component polyplex and the lipid/DNA weight ratio. The anti-CD3 antibody, the liposomal lipid and pH sensitivity were essential for transfection activity (Turner *et al*., 2002a).

Liposome in encapsulation of drug: Liposomal encapsulation of drugs represents a new drug delivery system that appears to offer important therapeutic advantages over existing methods of drug delivery (Wong *et al*., 1997). Liposome-encapsulated quinolones and specifically liposome-encapsulated ciprofloxacin dramatically enhances macrophage functions, induces NO production and augments the production of cytokines, rendering the composition an immunoprophylactic and immunotherapeutic agent with unique clinical potential. Liposome-encapsulated ciprofloxacin and other quinolones could be extremely useful in antimicrobial, anticancer and AIDS therapies. In such cases, the immunological status of the patient is often compromised or suppressed, making them susceptible to microbial infections and to the development of tumor growth. Selective augmentation of cellular immunity by activation of the microbicidal and tumoricidal activities of macrophages, induction of NO and cytokine production could be of primary importance to such patients in terms of protecting them against microbial infections and inducing their cellular host defense to tumor cells.

Liposome-encapsulated analgesic drugs also used in Pain management (Mezel, 2004). Liposome-encapsulated opioid analgesic agents delivered by the pulmonary route provide local, or systemic analgesia superior to that produced by the solution form of these agents administered by parentral (intravenous, intramuscular, or subcutaneous injection) or oral routes.

Liposomes in parenteral drug delivery systems: It has been reported that liposomes release their contents by interacting with cells in one of four ways adsorption, endocytosis, lipid exchange, or fusion (Ostro and Cullis, 1989). Liposome-entrapped drugs are distributed within the body much differently than free drugs; when administered intravenously to healthy animals and humans, most of the injected vesicles accumulate in the liver, spleen, lungs, bone marrow and lymph nodes. Liposomes also accumulate preferentially at the sites of inflammation and infection and in some solid tumors; however, the reason for this accumulation is not clear. Four major factors influence liposomes' *in vivo* behavior and bio-distribution:

- Liposomes tend to leak if cholesterol is not included in the vesicle membrane,
- Small liposomes are cleared more slowly than large liposomes,
- The half-life of a liposome increases as the lipid dose increases and
- Charged liposomal systems are cleared more rapidly than uncharged systems.

The most advanced application of liposome-based therapy is in the treatment of systemic fungal infections, especially with amphotericin B. Liposomal formulations of injectable antimicrobial agents and antineoplastic agents already are undergoing clinical testing and most probably will receive approval for marketing in the early 1990s.

The fate and disposition of intravenously injected liposomes depend on their physical properties (Thomas and Joseph, 2001) such as size, fluidity and surface charge. They may persist in tissues for hours or days, depending on their composition and half-lives in the blood range from minutes to several hours. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but the physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capilary endothelial, such as the sinusoids of the liver or spleen. Thus, this organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are highly sequestered in the liver and spleen. In general this in vivo behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow and lymphoid organs.

Liposome in cancer therapy: Liposome-based drug delivery is more efficient in breast cancer treatment (Park, 2002). This type of Drug delivery systems can provide enhanced efficacy and/or reduced toxicity for

anticancer agents. Long circulating macromolecular carriers such as liposomes can exploit the 'enhanced permeability and retention' effect for preferential extravasation from tumor vessels. Liposomal anthracyclines have achieved highly efficient drugencapsulation, resulting in significant anticancer activity with reduced cardiotoxicity and include versionswith greatly prolonged circulation such as liposomal daunorubicin and pegylated liposomal doxorubicin. Pegylated liposomal doxorubucin has shown substantial efficacy in breast cancer treatment both as monotherapy and in combination with other chemotherapeutics.

It has been occasionally noted that a powerful anti-cancer drug, especially one whose target is the cytoplasm or cell nucleus, does not work due to the low permeability across a plasma membrane, degradation by lysosomal enzymes through an endocytosis-dependent pathway and other reasons. Thus, several approaches using drug delivery systems (DDS) are focused on overcoming these difficulties, eventually leading to the induction of maximal ability of anti-cancer drug. In this respect, a new paradigm for cancer therapy using a novel drug delivery system fusogenic liposome (Kunisawa, 2005) was developed.

Fusogenic liposomes are composed of the ultraviolet-inactivated Sendai virus and conventional liposomes. Fusogenic liposomes effectively and directly deliver their encapsulated contents into the cytoplasm using a fusion mechanism of the Sendai virus, whereas conventional liposomes are taken up by endocytosis. Thus, fusogenic liposome is a good candidate as a vehicle to deliver drugs into the cytoplasm in an endocytosis-independent manner (Kunisawa, 2005).

Liposomal drug delivery systems provide stable formulation, provide improved pharmacokinetics and provide a degree of 'passive' or 'physiological' targeting to tumor tissue. However, these carriers do not directly target tumor cells. The design modifications that protect liposomes from undesirable interactions with plasma proteins and cell membranes and which contrast them with reactive carriers such as cationic liposomes, also prevent interactions with tumor cells. Instead, after extravasation into tumor tissue, liposomes remain within tumor stroma as a drug-loaded depot. Liposomes eventually become subject to enzymatic degradation and/or phagocytic attack, leading to release of drug for subsequent diffusion to tumor cells. The next generation of drug carriers under development features directs molecular targeting of cancer cells via antibody-mediated or other ligand-mediated interactions.

CONCLUSIONS

Liposomes are somewhat like cells in that they are round shells of *phospholipids*, the basic components of human cell walls. Enclosing a drug within a liposome entraps the drug as 'payload,' protecting it from early degradation within the body. Liposome encapsulation also improves a medication's *bioavailability* (the amount of time and specific distribution within the body), which can extend the treatment effect and reduce dosing.

Enclosing drugs within liposomes may dramatically alter the treatment of a variety of cancers by allowing for the improved delivery of anticancer medicines into the body. Because liposomal encapsulation radically alters a drug's absorption, distribution, metabolism and excretion (the treatment's *pharmacokinetics*), it can potentially address many of the current problems with controlled drug delivery. Glycoprotein or glycolipid cell surface components that play a role in cell-cell recognition, interaction and adhesion are used to overcome the limitation of liposomal drug targeting. Although the precise mechanism of their action is still unknown, they show potential in directing liposomes to particular cell types by their inclusion in the liposomal membrane. Potential therapeutic applications of liposomes include their use in the treatment of malignant tumors, lisozomal storage diseases, intracellular paracites, metal toxicities and diabetes mellitus. The liposome acts as the carrier of the active agent used in treatment of these conditions. Most of the applications involve intravenous injection of the liposomal preparations, but other routes of administration are conceivable. For example liposome entrapted insulin may offer some degree of protection of drug from gastric degradation and the possibility of GI absorption by endocytosis.

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