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Liposomes: A review

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ABSTRACT

Liposomes are microparticulate lipoidal vesicles which are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. Due to new developments in liposome technology, several liposome-based drug formulations are currently in clinical trial, and recently some of them have been approved for clinical use. Reformulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through alteration in their biodistribution. This review discusses the potential applications of liposomes in drug delivery with examples of formulations approved for clinical use, and the problems associated with further exploitation of this drug delivery system.

Keywords: Liposomes, ULV, SLV, Applications

INTRODUCTION

Liposomes have been receiving a lot of interest as a carrier for advanced drug delivery.[1] Liposomes were first produced in England in 1961 by Alec D. Bangham, who was studying phospholipids and blood clotting.[2] 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 hydrophobic ends; fat-soluble medications were incorporated into the phospholipid layer.

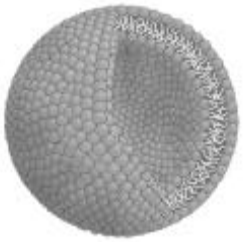

Fig 1. Spherical Formation of Liposomes

A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chain like egg phosphatidylethonalimine or of pure components like DOPE (dioleolylphosphatidylethanolamine).
The lipid bilayer can fuse with other bilayers (eg. 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 dilevered past the lipid bilayer.

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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.

TYPE OF LIPOSOMES

Depending upon the structure there are two type of liposomes.[3]

a) Unilamellar liposomes: Unilamellar vesicles has a single phospholipid bilayer sphere enclosing aqueous solution.

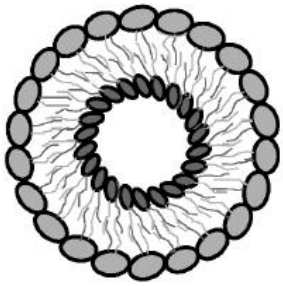

Fig 2. Very Small, Single Layer liposome

b**) 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 phospholipid spheres separated by layers of water.

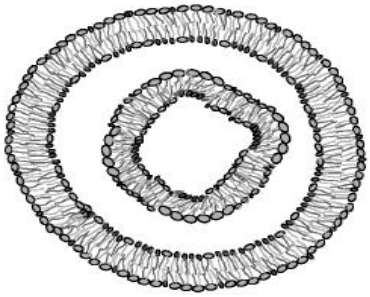

Fig 3. Large Vesicle, Multilayer Liposme

COMPOSITION AND CHARACTERSTICS OF LIPOSOMES

Usually liposomes composed of cholysterol and phospholipids. The structure, composition and proportion being practically the same as in the host cell membranes.[2] The phospholipids possess a hydrophobic tail structure and a hydrophilic head componrnt and organize in the following 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 4.). In this way, double lipid layers are formed which seal off to form small vescicles similar to the body cells and oraganelles. These sphere or liposomes constitute small deposits that can be made to contain an antigen, an antibiotic, an allergen, a drug substance or a gene. The liposomes can in turn be introduced in the body without trigrring immune rejection reaction. Phospholipid bilayers are the core structure of liposomes and cell membrane formation.

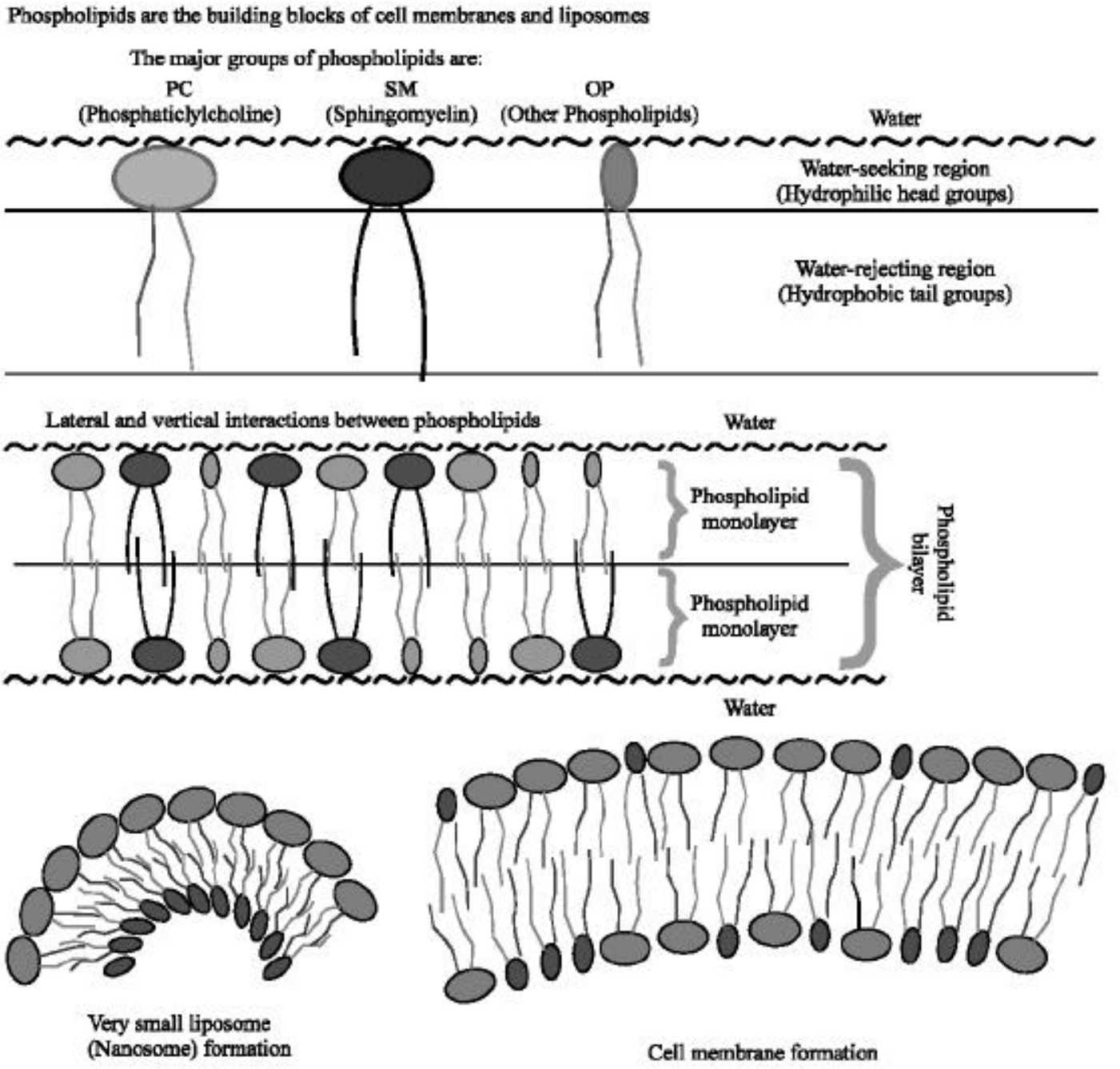

Fig 4. Compositional Structure of Liposome

MECHANISM OF TRANSPORTATION THROUGH LIPOSOMES

Liposome can interact with cells by four different mechanism[2]

- 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.

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

LIPOSOME PREPARATION

a) Handshaking Method

In order to produce liposome lipid molecules must be introduced into an aqueous environment. When dry lipid layer film is hydrated the lamellae swell and grow into myelin figures. Only mechanical agitation provided by vortexing, shaking, swering or pippeting causes myelin figures to break and reseal the exposed hydrophobic edges resulting in the formation of liposomes can be made by hand shaken method.

b) 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 sonicator is directly immersed into the liposome 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 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 Sonicator

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 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 liposomes can fuse with other bilayers, thus delivering the liposome contents. By making liposomes in a solution of DNA or drug they can be delivered past lipid bilayer.

c) Reverse Phase Evaporation Method

Historically this method provided a break through 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 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 collapse and some of the inverted micelles into a gel like and viscous state. At a critical point in this procedure, the gel state collapse 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. Liposome made by this method can be made from various lipid formulations and have aqueous volume to lipid ratios that are four time higher than multi lamellar liposomes or hand shaken method.

d) Freeze Dried Rehydration Method

Freeze dried liposomes are formed from preformed liposomes. Very high encapsulation efficiencies even for macromolecules can be achieved using this method. During the dehydration the lipid bilayers and the material 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 is should be done very carefully. The aqueous phase should be added in very small portions with a micropippete 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.

LIPOSOMES AS A DRUG DELIVERY SYSTEM

Liposomal vesicles were prepared in the early years of their history from various lipid classes identical to those present in most biological membranes. Basic studies on liposomal vesicles resulted in numerous methods of their preparation and characterization. Liposomes are broadly defined as lipid bilayers surrounding an aqueous space. Multilamellar vesicles (MLV) consist of several (up to 14) lipid layers (in an onion-like arrangement) separated from one another by a layer of aqueous solution. These vesicles are over several hundred nanometers in diameter. Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25–50 nm (according to some authors up to 100 nm) in diameter. Large unilamellar vesicles (LUV) are, in fact, a very heterogenous group of vesicles that, like the SUVs, are surrounded by a single lipid layer. The diameter of these liposomes is very broad, from 100 nm up to cell size (giant vesicles).[4] Besides the technique used for their formation the lipid composition of liposomes is also, in most cases, very important. For some bioactive compounds the presence of net charged lipids not only prevents spontaneous aggregation of liposomes but also determines the effectiveness of the entrapment of the solute into the liposomal vesicles. Natural lipids, particularly those, with aliphatic chains attached to the backbone by means of ester or amide bonds (phospholipids, sphingolipids and glycolipids) are often subject to the action of various hydrolytic (lipolytic) enzymes when injected into the animal or human body. These enzymes cleave off acyl chains and the resulting lysolipids have destabilising properties for the lipid layer and cause the release of the entrapped bioactive component(s). As a result new types of vesicles, that should merely bear the name of liposomes as their components are lipids only by similarity of their properties to natural (phospho)lipids, have been elaborated. These vesicles, still named liposomes, are made of various amphiphile molecules (the list of components is long). The crucial feature of these molecules is that upon hydration they are able to form aggregation structures resembling an array and have properties of natural phospholipid bilayers.

Table1. Example of drugs in liposomal formulation

Drug	Application	Commercial Name	Composition of Liposomes
Amikacin	Bacterial Infection	MiKasome	HSPC/CH/DSPG
Adriamycin	Stomach Cancer	-	DPPC/CH
Ampicillin	Listeria monocytogenesis	-	CH/PC/PS
Annamycin	Breast Cancer,Leukemia	Annamycin	Liposomes
Amphotericin B	Systemic Fungal Infection	AmBisome	HSPC/CH/DSPG
All-*trans*retionic acid	Prostate Cancer, Leukemia	ATRAGEN	Liposomes
Muramyl dipeptide	Immunostimulator	-	DSPC/PS 1:1
Ciprofloxacin	*Pseudomonas aerogonisa*	-	DPPC
Clodronate	Macrophage suppresion	-	PC/CH
Cyclosporin	Immunosuppresor	-	PC/CH
Chloroquinine	Malaria	-	PC/PG/CH
Doxorubicin	Cancer	Doxil	HSPC/CH/PEG
Daunorubicin	Breast Cancer	Daunaxome	DSPC/CH
Gangiciclovir	HSV	-	Liposomes
Intralukin-2	Immunostimulant	-	DMPC
Mitoxantron	Colon Cancer	-	PC/CH
Methorexate	Cancer	-	DPPC/PI
Nystatin	Fungal Infection	Nyotran	Liposomes
Pentostam	Leishmanioses	-	Niosomes
Cisplastin	Mezotelioma	PLATAR	Liposomes
Lurotecon	Cancer	NX211	Liposomes
Prostaglandanin	Antiinflammatory	-	Liposomes
Ribavirin	Herpes Simplex	-	PC
Streptosotocin	Lymphocyte activator	-	DMPC/CH
Suramin	Trypanosomes	-	DPPC

Among such molecules, various amphiphiles have been employed, such as ether lipids, fluorinated lipid, synthetic double chain amphiphiles as well as single chain amphiphiles, such as N-alkylindoles, polyhydroxyl lipids, polyhedral non-ionic surfactants, polymerized liposomes, cationic amphiphiles, plasmalogens and others. This resulted in various new formulations of vesicle compositions and new names given to them, such as niosomes, lederosomes, archeosomes, etc.

The common feature of classical liposomes, i.e., made preferentially of phospholipids, and of vesicles made of amphiphilic molecules, was their ability to form dynamic lamellar structures with barrier properties separating the interior of the vesicles from the outside medium. One may conclude that, at present, the term "liposomes" covers not only phospholipid-based vesicles but also other vesicular structures with properties identical or similar to those of classical, natural phospholipid based liposomes.

In the early 70's the use of liposomes as a drug carrier system was proposed by Gregoriadis & Ryman.[5] Since this first report, liposomes were developed as an advanced drug delivery vehicle. They are generally considered non-toxic, biodegradable and non-immunogenic. Associating a drug with liposomes markedly changes its pharmacokinetics and lowers systemic toxicity; furthermore, the drug is prevented from early degradation and/or inactivation after introduction to the target organism.[6-8]

The use of liposomes or, in general, vesicular structures for the delivery of various active compounds is recognized in relation to water solubility of the compound. When the compound is water soluble, the size and volume of the aqueous compartment of the vesicle is crucial. In contrast, hydrophobic compounds will prefer incorporation into the lipid (amphiphile) layer that constructs the vesicle. In such a case, the size of the aqueous compartment is not important. Depending on the need, one can use SUV type or MLV type vesicles for effective entrapment and delivery of the drug to the target tissues or cells.

Nevertheless, charge properties and interactions of the active compound with vesicle forming molecules will determine the affectivity of entrapment, i.e., the amount of the compound that can be "loaded" into a single vesicle. On the other hand, the composition of the molecules used for the formation of the vesicular structure will, at least, affect the fate of vesicles from the site of their introduction as well as the interaction with components of the body (e.g., surface charge, serum proteins, lipoproteins, opsonin system, phagocytic system and finally target cells). In the earlier studies, when therapeutically active substances were not easily available, most of the experiments were done using a marker compound. The results, however, were not the same as those obtained in experiments in which an active substance was used and the conditions were more related to the real situation (*ex vivo*, *in vivo*). These findings implicate the necessity for studies in which an active substance is used and the conditions of the experiments resemble, as closely as possible, those of therapeutic liposomal (vesicular) drug application. The benefits of liposomal formulations were already demonstrated clinically and stimulate many laboratories (research and pharmaceutical) in their efforts to introduce new liposomal/vesicular drugs. These can be illustrated with the data presented in Table 1.

PPROSPECTS

Further advances in liposome research have been able to allow liposomes to avoid detection by the body's immune system, specifically, the cells of reticuloendothelial system (RES). These liposomes are known as "stealth liposomes", and are constructed with PEG (Polyethylene Glycol) studding the outside of the membrane. The PEG coating, which is inert in the body, allows for longer circulatory life for the drug delivery mechanism. However, research currently seeks to investigate at what amount of PEG coating the PEG actually hinders

binding of the liposome to the delivery site. In addition to a PEG coating, most stealth liposomes also have some sort of biological species attached as a ligand to the liposome in order to enable binding via a specific expression on the targeted drug delivery site. These targeting ligands could be monoclonal antibodies (making an immunoliposome), vitamins, or specific antigens. Targeted liposomes can target nearly any cell type in the body and deliver drugs that would naturally be systemically delivered. Naturally toxic drugs can be much less toxic if delivered only to diseased tissues. Polymersomes, morphologically related to liposomes can also be used this way.

CONCLUSION

It was concluded from the review that liposomes have great potency in drug delivery system. Drug of both category (hydrophilic/ lipophillic) easily embedded in the liposomes. The drug was delivered in the body in the controlled manner or wants to be site specific. In many hard diseases (cancers, tumors, HIV) the drug was easily and effective delivered by the means of liposomes.

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