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REVIEW ARTICLE

Liposomal Drug Delivery System: An Overview

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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.

Key words: Liposomes, MLV, 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.

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. 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 delivered past the lipid bilayer (**Fig 1**).

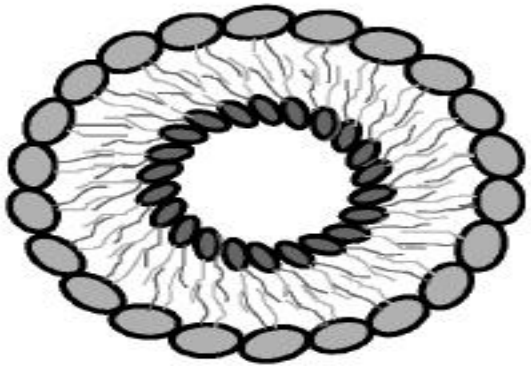

Fig 1: Very Small, Single Layer liposome

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 2).**

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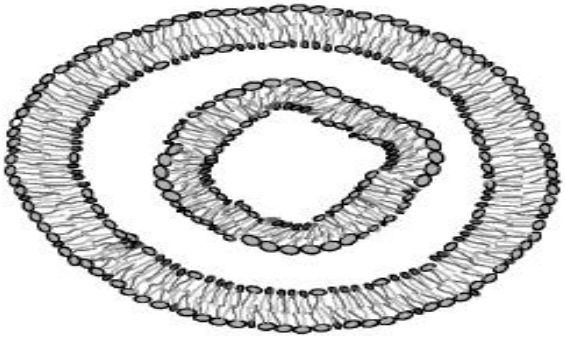

Fig 2: Large Vesicle, Multilayer Liposome

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 component 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 3**). In this way, double lipid layers are formed which seal off to form small vescicles similar to the body cells and oraganelles. 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. The liposomes can in turn be introduced in the body without triggering immune rejection reaction. Phospholipid bilayers are the core structure of liposomes and cell membrane formation.

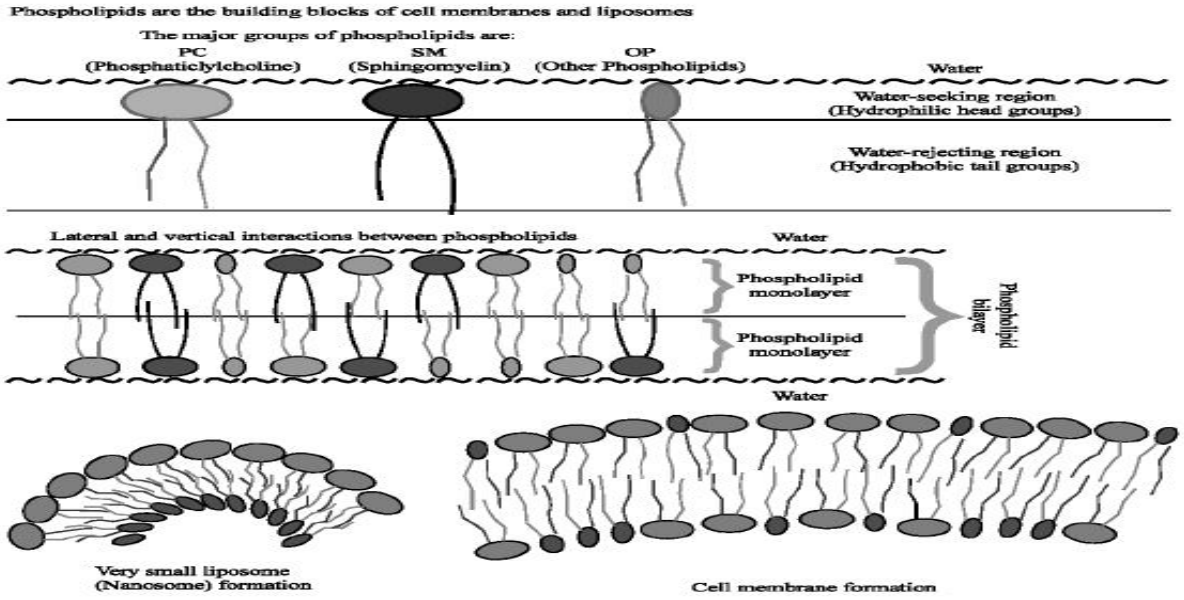

Fig 3: Compositional Structure of Liposome

MECHANISM OF TRANSPORTATION THROUGH LIPOSOMES

Liposomes can interact with cells by four different mechanisms[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.

METHODS OF PREPARATION OF LIPOSOMES

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[4].

(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. The main drawback of this method is the exposure of the materials to be encapsulated to organic solvent and to sonication[5,6].

(ii) Solvent Spherule Method

A method for the preparation of MLVs of homogeneous size distribution was proposed by Kim et al. 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[7].

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[8].

(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. 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) [9].

C) Large Unilamellar Liposomes (LUV)

They have high internal volume/encapsulation efficiency and are now a 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[10].

(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[11].

(ii) Detergent Removal Methods

The detergents at their critical micelle 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 [12]. 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 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[13] (b) by adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-2[14] (c) by binding of octyl glucoside (a detergent) to Amberlite XAD-2 beads[15].

(iii) Reverse 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[16]. 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 has also been modified to entrap plasmids without damaging DNA strands[17].

(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. The main advantage of this method is that macromolecules can be 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[18].

(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 yield 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 MLVs were prepared by these were passed through a Microfluidizer (Microfluidics Corporation, Newton, MA, USA) at 40 psi inlet air pressure. The size range was 150-160 nm after 25 recylces. In the Microfluidizer, the interaction of fluid streams takes place at high velocities (pressures) in a precisely defined microchannels 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[19].

(vi) 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. 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[20,21,22].

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 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)[23].

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.

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.[24] 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.[25-27]

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.

PPROSPECTS

Further advances in liposome research has been able to allow liposomes to avoid detection by the body's immune system, specifically, the cells of reticulo endothelial 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 severe diseases (cancers, tumors, HIV) the drug was easily and effective delivered by the means of liposomes.

REFERENCES

1. Langer R. Drugs on Target. Science 2001; 293: 58-59.
2. Bangham AD. Liposomes. 1st ed., New York: Marcel Dekker; 1983. p. 1-26.
3. Thomas WL, Joseph RR. Remington: The Science and Practice of Pharmacy. 20th ed., Pennsylvania: Mack Publishing Company; 2001. p. 18042.
4. Bangham A.D., Standish M.M., Watlins J.C. J. Mol. Biol. 1965; 13: 238.
5. Papahadjopoulos D., Watkins J.C. Biochem. Biophys. Acta. 1978; 135: 639.
6. Gruner S.M., Leak R.P., Janoff S., Ostro M.J. Biochem. 1985; *24*: 2833.
7. Kim S., Jacobs R.E., White S.H. Biochem. Biophys. Acta. 1985; 812: 793.
8. Oezden M.Y., Hasirci V.N. Biochem. Biophys. Acta. 1991; 1075: 102.
9. Hamilton R.L., Guo L.S.S. Liposome Technology. Gregoriadis G. ed., Florida: CRC Press 1984; 1: 37-49.
10. Deamer D., Bangham A.D. Biochem. Biophys. Acta. 1976; 443: 629.
11. Batzri S., Korn E.D. Biochem. Biophy. Acta. 1973; 298: 1015.
12. Kagawa Y., Racker E., J. Biol. Chem. 1974; 246: 5477.
13. Enoch H.G., Strittmatter P., Proc. Natl. Acad. Sci. USA. 1979; 76: 145.
14. Gerritson W.J., Verkley A.J., Zwaal R.F.A., van Deenan L.L.M., Eur. J. Biochem. 1978; 85: 255.
15. Philippot J.R., Mutafschicv S., Liautard J.P. Biochem. Biophys. Acta. 1985; 821:79.
16. Szoka F.J., Papahadjopoulos D. Proc. Natl. Acad. Sci. 1978; 75: 4194.
17. Naga N., Yagi K. J. Clin. Biochem. 1989; 7: 175.
18. Papahadjopoulos D., Vail W.J. Ann. N.Y. Acad. Sci. 1978; 308: 259.
19. Riaz M., Weiner N., Martin F. Pharmaceutical Dosage Forms: Disperse Systems. Lieberman H.A., Reiger M.M. And Banker G.S., eds., New York: Marcel Dekker; 1989. p. 567.
20. Pick U. Arch. Biochem. Biophys. 1981; 212: 186.
21. Ohsawa T., Miura H. And Harada K. Chem. Pharm. Bull. 1985; 33: 3945.
22. Liu L., Yonatan T. J. Microencapsulation. 1994; 11: 409.
23. Woodle M.C., Papahadjopoulos D. Liposome preparation and size characterization methods. Enzymol. 1989; 171: 193-217.
24. Gregoriadis G., Ryman B.E. Lysosomal localization of fructofurano side containing liposomes injected into rats. Biochem J. 1972; 129: 123-133.
25. Allen T.M. Liposomes: Opportunities in drug delivery. Drugs. 1997; 54: 8-14.
26. Allen T.M., Moase E.H. Therapeutic opportunities for targeted liposomal drug delivery. Adv Drug Deliv Rev. 1996; 21: 117-133.
27. Bally M., Nayar R., Masin D., Hope M.J., Cullis P.R., Mayer L.D. Liposomes with entrapped doxorubicin exhibit extended blood residence times. Biochem Biophys Acta. 1990; 1023: 133-139.