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Review Article

LIPOSOMES AS A DRUG DELIVERY CARRIER - A REVIEW

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ABSTRACT
The evolution of the science and technology of liposomes as a drug carrier has passed through a number of distinct phases. Because they exhibit peculiar properties due to their structure, chemical composition amphiphile nature, physico-chemical characters and colloidal size, which are used in various applications. These properties point to several applications as the solubilizer for insoluble drugs, dispersants, and sustained release system, delivery system for the encapsulated substance, stabilizer, protective agents, and micro reactive being the most obvious ones. Yet interest in liposomes, especially among academic workers, spread rapidly we attribute this to the remarkable structural versatility of the system, which enables the design of countless liposomes versions to satisfy particular needs in terms of both technology and optimal function in vivo.

KEYWORDS: Liposomes, Lamellar vesicles.

INTRODUCTION

Liposomal vesicles were prepared in the early years of their history from various lipid classes identical to those present in most biological membranes.[1] Liposomes were discovered in the mid of 1960' s and originally studied as cell membrane model Paul Ehrlich coined the term "magic bullet" in 20th century where carrier system's was proposed to simply carry the drug to its of action and releasing its selectively while non target sites should absolutely be exempted from drug effect.[2] The exploration and progressive advent of liposomal drug delivery system has rekindled interest in magic bullet approach, for surely man's ingenuity can find means for directing these drugs filled packed or lipid bilayer vesicles to specific cell or anatomical sites within the body.[3] Liposomes were described as a model of cellular membranes and quickly were applied to the delivery of substances to cells. Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated. When mixed in water under low shear conditions, the phospholipids arrange themselves in sheets, the molecules aligning side by side in like orientation, "heads" up and "tails" down. These sheets then join tails-to-tails to form a bilayer membrane (Figure .1), which encloses some of the water in a phospholipids sphere.[4]

MECHANISM OF LIPOSOMAL FORMATION

Liposomes are formed open hydration of lipid molecules normally lipids are hydrated from a dry state (thin or thick lipid film, spray dried powder), and stacks of crystalline bilayers become fluid and swell myelin-long, thin cylinders grow and upon agitation detach self close in to large, multilameller liposomes because this eliminates unfavorable interactions at the edges. Once the large particles are formed they can be either broken by mechanical treatment in to smaller bilayered fragments, which close into smaller liposomes. The size of liposomes in the budding off mechanism is very difficult to calculate, in the self closing bilayer mechanism the liposomes size depends, the bending elasticity of the bilayer and the edge interactions of open fragments. These factors determine the size of the vesicle size (figure. 2).[5]

CLASSIFICATION OF LIPOSOMES

Multilameller 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 heterogeneous 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).[6] Besides the technique used for their formation the lipid composition of liposomes is also, in most cases, very important.[7]

METHODS OF LIPOSOMES PREPARATION

Basic studies on liposomal vesicles resulted in numerous methods of their preparation and characterization.[8,9]

A. Hydration stage

1. Mechanical methods
    - Vortexing or hand shaking of phospholipid dispersions (MLV)
    - 'Microfluidizer' technique (mainly SUV)
    - High-shear homogenization (mainly SUV)
2. Methods based on replacement of organic solvent(s) by aqueous media
    - Removal of organic solvent(s) before hydration (MLV, OLV, SUV)
    - Reverse-phase evaporation (LUV, OLV, MLV)
    - Use of water immiscible solvents: ether and petroleum-ether infusion (solvent vaporization) (MLV, OLV, LUV)
    - Use of water miscible solvents such as ethanol injection (MLV, OLV, SUV)
3. Methods based on detergent removal
    - Gel exclusion chromatography (SUV)
    - 'Slow' dialysis (LUV, OLV, MLV)
    - Fast dilution (LUV, OLV)
4. Methods based on size transformation and fusion
    - Spontaneous fusion of SUV in the gel phase (LUV)
    - Freeze-thawing (MLV)
    - Freeze-drying (MLV)

B. Sizing stage

1. High pressure extrusion
2. *Low pressure extrusion*
3. Ultrasonic treatment

C. Removal of non-encapsulated material

1. Dialysis
2. Ultracentrifugation
3. Gel-permeation chromatography
4. Ion-exchange resins

TARGETING VIA LIPOSOMES

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.[6] Nevertheless, charge properties and interactions of the active compound with vesicle forming molecules will determine the effect of entrapment, i.e., the amount of the compound that can be "loaded" into a single vesicle.[10] 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 component 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.[11] 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.[12]

Liposomes are well established as drug carriers in topical treatment of diseases, especially in dermatology. They can enhance penetration of encapsulated hydrophilic drugs into the skin to enable a proper therapeutic effect. Because of they are able to carry with them any enclosed substances into the dermis and to 'the individual cells.[13]

SUSTAINED RELEASE OF THE INCORPORATED DRUG

Liposomes are typical vehicles, which are able to transport dermatological and cosmetic active agents of different types. The active agents are encapsulated und protected against environmental influences. Liposomes spread out excellently in the horny layer of the skin and form depots of active agents. Aqueous dispersions show a clear to milky appearance according to the size of the Liposomes. Similar to the horny layer of human skin, liposomes consist of one or several bilayers of phosphatidylcholine. Liposomes without active agents ("empty liposomes") show all dermatological and cosmetic effects of phosphatidylcholine.[14]

The size of these spheres is very small, in the order of a nanometer. As illustrated, the spheres are hollow inside and enclose some of the liquid material in which they were formed (inclusion). Because of the small size of the phospholipid molecule and microspheres, they can pass through the epidermis and act as a carrier for the enclosed substances.

RELEASE KINETICS OF LIPOSOMAL PAYLOAD

Liposomes are most useful for being able to transfer and deliver active ingredients to the application site of formulation. The liposome wall is very similar, physiologically, to the material of cell membranes. Application of formulation over skin area causes deposition of liposomes on the skin and begins to merge with the cellular membranes. In the process, the liposomes release their payload of active materials into the cells. As a consequence, not only is delivery of the actives very specific directly into the intended cells but also the delivery takes place over a longer period of time. Liposomes exhibit better stability, penetration and efficacy at lower usage levels.[12]

Liposomes as a delivery system can be made to release their payload under a variety of conditions.

- Slow / Fast Release of Hydrophilic Payload
- Slow / Fast Release of Hydrophobic Payload
- Bilayer Composition
    1. Chain Length
    2. Saturation
    3. Lipid Class
- Physical Configuration of Liposome
- Solvent-Dependent Release
- pH-Dependent Release
- Temperature-Dependent Release

APPLICATIONS OF LIPOSOMES

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. Since this first report, liposomes were developed as an advanced drug delivery vehicle. They are generally considered non-toxic, biodegradable and non-immunogenic.[15] 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.[10] 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.[16] 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.

Liposomes in human therapy

Despite of the good and encouraging results obtained using liposomes as vehicles for drugs in numerous diseased animal models, in human therapy; the use of liposomes is restricted to systemic fungal infections and cancer therapy, only. However, liposomes based vaccines show great promise and a vaccine against hepatitis A is already on the market.

Liposomes in anticancer therapy

Based on the early studies that showed that encapsulation of a drug inside of liposomes reduces its toxic side effects, the liposomes were considered as attractive candidates for the delivery of anticancer agents. However, their use was hampered by the rapid uptake of conventional liposomes by MPS cells. The increase of in vivo circulation time of modified lipids (PEG polymerized lipids, gangliosides, shingomyelin etc.) restored the initial expectation of the advantages of liposomes. Intravenously administered stealth liposomes were passive targeted to solid tumors due to their extravasation in leaky blood vessels supporting the tumor[24]. The good results obtained with liposomal-encapsulated doxorubicin and daunorubicin have lead to two products licensed for use in the treatment of Kaposi' sarcoma, namely Doxil and Daunoxome. Doxil (commercialized by Sequus Pharmaceuticals, Menlo Park, USA) is a suspension of doxorubicin precipitated in 80-100 nm sterically stabilized liposomes. Daunoxome (commercialized by NeXstar Pharmaceuticals, Inc., Boulder, USA) is a small, rigid formulation of liposomes with daunorubicin. These liposomes circulate in the vasculature of patients for several days, and thus have increased chances of extravasating at sites of increased permeability. The success achieved with anthracycline anticancer agents led to the development of other liposomal formulations that are in preclinical stages (5-fluorouracil lipid analogue[17], vincristine[18] a porphyrin derivative for use in combination with laser light irradiation, bleomycin[19], paclitaxel[20], valinomycin in combination with cisplatin.[21]

Liposomes in infection treatment

Due to their uptake by the cells of the MPS, mainly Kupffer cells and spleen macrophages, conventional liposomes are useful in the treatment of parasitic infections of the MPS, such as leishmaniasis. Encapsulating the amphotericin B into liposomes reduces the renal and general toxicity, and the therapeutic efficiency is improved. Ambisome is a formulation of small, negatively charged liposomes with amphotericin B licensed for clinical use and commercialized by NeXstar, Pharmaceuticals Inc., Boulder, USA. Now, the attention is focused on the encapsulation of more powerful antibiotics (that are exceedingly toxic in free form) and on the develop development of liposomal formulations for delivering the drugs to other sites than MPS. The encapsulation of the anti-tuberculosis drug rifampicin or isoniazid in liposomes targeted to lung improves the efficacy of the drug.[22]

Liposomes as vaccine system

Liposomes can be used as enhancers of the immunological response by incorporation of antigens 61. For this purpose the liposomes are administered intramuscularly, a location where the encapsulated antigen is released slowly and accumulate passively within regional lymph nodes. To control the antigen release and to improve the antibody response, the liposomes encapsulating antigens are subsequently encapsulated into alginate lysine microcapsules.[23] At present, Epaxal, a liposome-based vaccine against hepatitis A was licensed for clinical use and was introduced on the market by Swiss Serum and Vaccine Institute, Bern, Switzerland.[24] This vaccine contains formalin inactivated hepatitis A virus particles attached to phospholipid vesicles together with influenza virus haemagglutinin. Hepatitis A virus incorporated into liposomes proved to be a suitable formulation in term of rapid seroconversion, high level of mean antibody content and low reactogenicity.[25] Also, there is in clinical trial vaccines against influenza, hepatitis B, diphtheria, tetanus, E-coli infection.

Liposomes in gene delivery

Gene therapy is the process which DNA delivers sequences encoding specific altered genes to cells with the goal of treating or curing genetic diseases. Thus, instead of treating the symptoms of the disease, as in conventional medicines, gene therapy has the potential to correct the underlying cause of genetic diseases. While the idea of gene therapy is a simple concept, the delivery of genes to the diseased areas turned out to be a difficult task. The problems associated with the use of viral vectors for gene therapy, lead to the search for less-hazardous, nonviral delivery systems. As an alternative to viral vectors, cationic liposomes

have been developed for gene transfer since they have no limit for the size of the genes to be delivered and exhibit low immunogenicity. The efficacy of this system has been limited by the non-specific adherence to many cell types. In order to obtain an effective DNA transfer it is necessary to administer liposomes to a site near to the target area. The use of ligand-targeted liposomes will make possible to direct them precisely to diseased cells and not to other cells. Some pharmaceutical companies (Vical Company, San Diego, USA, Targeted Genetics Corporation, Seattle, USA, and ValentisR R Burlingame, USA) are engaged in liposome- based gene delivery and have products in clinical trials. Vical Company (San Diego, USA) has two compounds on trial that are based on liposomes for gene delivery: (i) Allovectin-7: liposomes carrying a gene for HLA-B7 (a highly immunogenic molecule) that are injected into tumors: this is in phase III trial for metastatic melanoma and phase II trial for patients with head and neck squamous cell carcinoma and (ii) Leuvectin, a DNA/lipid complex containing gene for IL-2, a immunostimulatory cytokine, that is in phase II trial for patients with prostate cancer. ValentisR Company (Burlingame, USA) has a liposome-based system in phase I trial for gene therapy with Del-1 (Developmentally Regulated Endothelial Locus-1, an extracellular matrix protein involved in the early growth and development of blood vessels and bone) for the treatment of peripheral arterial disease and ischemic heart disease.

A cationic liposome /E1A complex (a gene from common cold virus that acts as tumor inhibitor) that is injected intratumoral, is under investigation by Targeted Genetics Corporation (Seattle, USA); the liposome complex is in phase II study in the treatment of patients with recurrent head and neck squamous cell carcinoma[26] and in phase I in ovarian cancer in combination with paclitaxel (Taxol) and cisplatin chemotherapy.[27] Intratumoral injections of liposome/ E1A complex were safe and well tolerated. The E1A gene expression was accompanied by HER- 2/neu down regulation, increased apoptosis, and reduced proliferation. Also in clinical trials, the UK CF Gene Therapy Consortium (London, United Kingdom) employs the complexes liposome/gene CFTR (cystic fibrosis transmembrane conductance regulator) that is delivered as an aerosol to the nose and lung of patients with cystic fibrosis.[28]

ADVANTAGES OF LIPOSOMES

- Controlled drug delivery system
- Biodegradable, non toxic
- Carry both water soluble and oil soluble drugs
- Prevention of oxidation
- Protein stabilization
- Controlled hydration.

Liposomes are most useful for being able to transfer and deliver ingredients to the application site. The liposome wall is very similar, physiologically to the material of cell membranes. Liposome delivers actives very specific directly in to the intended cells.

The basic aspects of the liposomal drug delivery system are

- ***Protection*:** Active materials are protected by virtue of membrane barrier function
- ***Sustained release:*** Such release is dependent on the ability to vary the permeability characteristics of the membrane by control of bilayer composition and lamelarity.
- ***Controlled release:*** Drug release enabled by exploiting lipid phase transition in response to external triggers such as changes in temperature or pH.
- ***Targeted delivery:*** Liposomes size and surface charge to effect passive delivery to body organs or by incorporating antibodies or other ligands to aid delivery to specific cell types.

CONCLUSION

Liposomes have been realized as extremely useful carrier systems, additive(s) and tools in various scientific domains. Thus, liposomes over the years have been investigated as the major drug delivery systems due to their flexibility to be tailored for varied desirable purposes. The flexibility in their behavior can be exploited for the drug delivery through any route of administration and for-any drug or material irrespective of its physicochemical properties. The uses of liposomes in the delivery of drugs and genes to tumor sites are promising and may serve as a handle for focus of future research**.**

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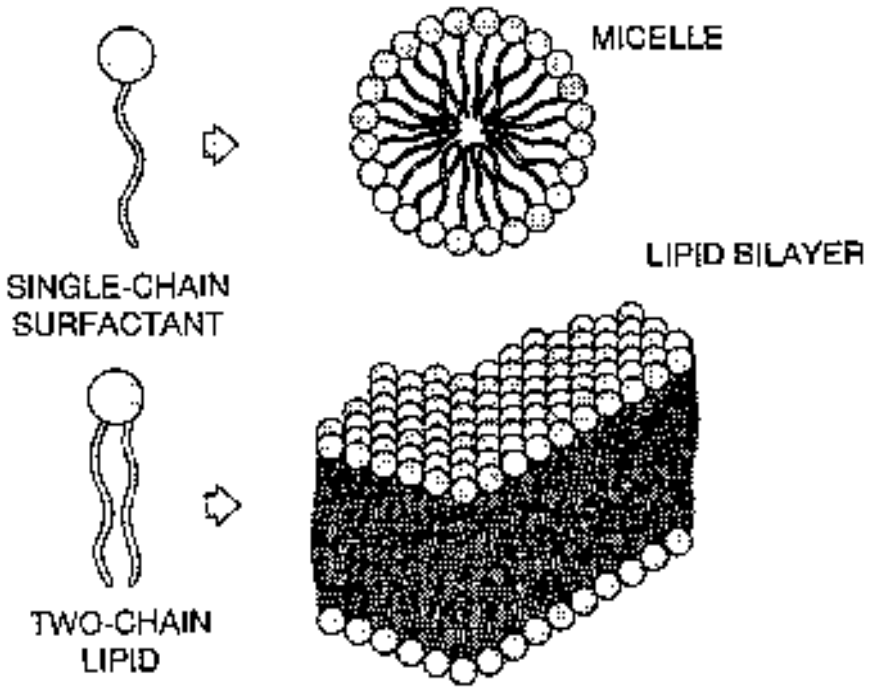

Figure 1: Structure of Micelle and lipid bilayers

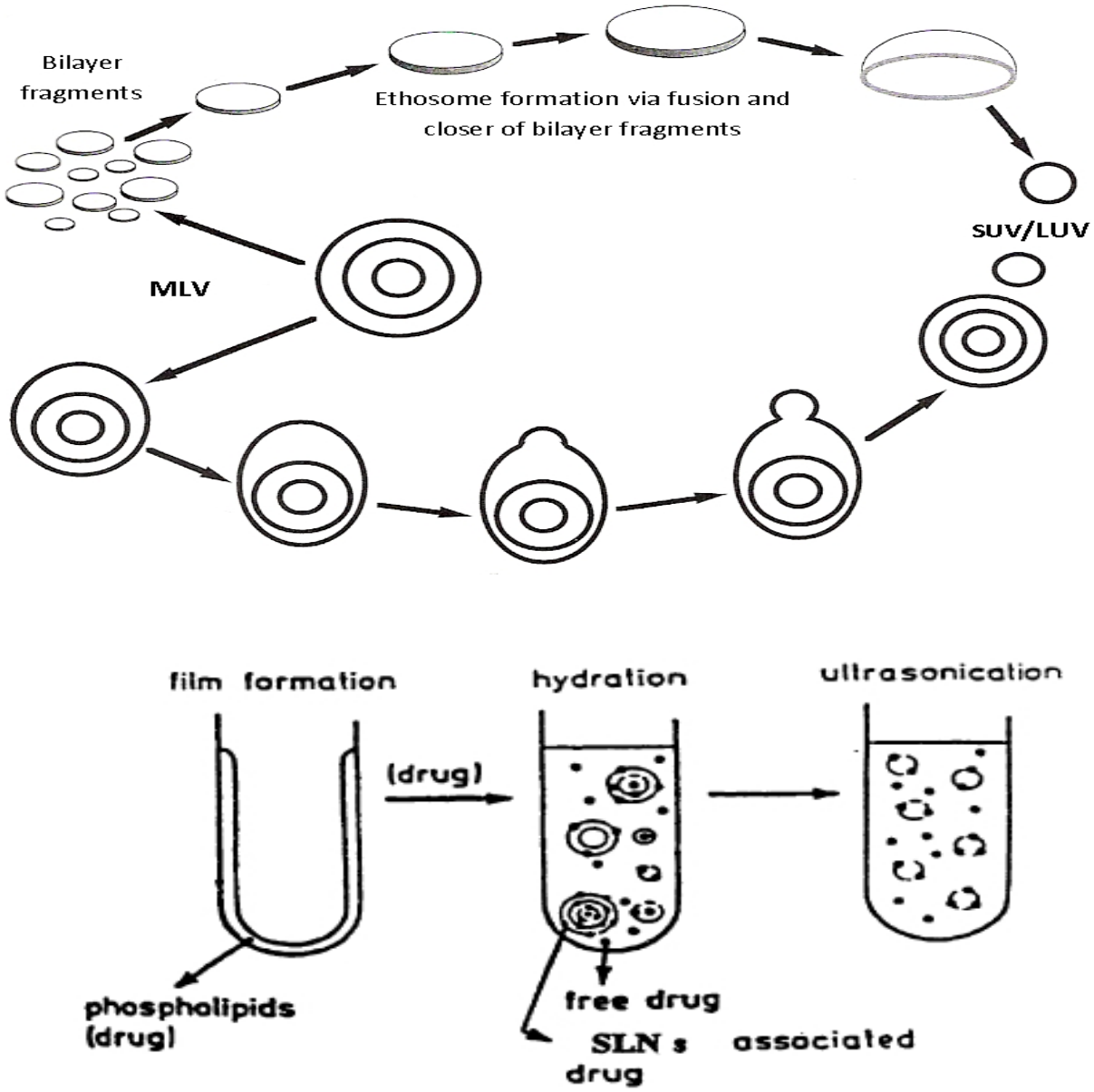

Figure 2: Mechanism of liposome formation