LIPOSOMES: CLINICAL ASPECT AND CHALLENGES

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ABSTRACT

A liposome is a spherical vesicle having at least one lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs. Liposomes have been used to improve the therapeutic index of new or established drugs by modifying drug absorption, reducing metabolism, prolonging biological half-life or reducing toxicity. Once lipids are hydrated in the presence of hydrophilic drugs, a portion of the drug gets entrapped inside the liposome and another portion remains in the bulk, outside the aqueous core of the liposome. Hydrophobic drugs are solubilized in the phospholipid bilayer of the liposomes that mainly provide a hydrophobic environment. Liposome stability can be explained by physical, chemical and biological means which are all interrelated. Bearing in mind the susceptibility of liposomes to the previously mentioned physical and chemical degradation mechanisms, the conditions required in conventional sterilization techniques (except filtration) are rather concerning since they involve the usage of heat, radiation and/or chemical sterilizing agents. Among all the nanomedicine platforms, liposomes have demonstrated one of the most established nanoplatforms with several FDA-approved formulations for cancer treatment, and had the greatest impact on oncology to date, because of their size, biocompatibility, biodegradability, hydrophobic and hydrophilic character, low toxicity and immunogenicity.

Keyword: Liposome, Therapeutic Index, Toxicity, Nanomedicine, FDA, Oncology

INTRODUCTION

A liposome is a spherical vesicle having at least one lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutica drugs.

Liposomes can be prepared by disrupting biological membranes (such as by sonication). Liposomes are most often composed of phospholipids, especially phosphatidylcholine, but may also include other lipids, such as egg phosphatidylethanolamine, so long as they are compatible with lipid bilayer structure. A liposome design may employ surface ligands for attaching to unhealthy tissue [1].

Liposomes were first described by British haematologist Alec D Bangham in 1961 (published 1964), at the Babraham Institute, in Cambridge [2]. Liposomes have been used to improve the therapeutic index of new or established drugs by modifying drug absorption, reducing metabolism, prolonging biological half-life or reducing toxicity. Drug distribution is then controlled primarily by properties of the carrier and no
longer by physico-chemical characteristics of the drug substance only.

Liposomes are composite structures made up of phospholipids and may contain small amounts of other molecules. Though liposomes can vary in size from low micrometer range to tens of micrometers, unilamellar liposomes are typically in lower size range with various targeting ligands attached to their surface allowing for their surface-attachment and accumulation in therapeutic efficiency of drugs in preclinical models and in humans compared to conventional formulations due to the alteration of biodistribution. Liposome binding drugs, into or onto their membranes, are expected to be transported without rapid degradation and minimum side effects to the recipient because generally liposomes are composed of biodegradable, biologically inert and non-immunogenic lipids. Moreover, they produce no pyrogenic or antigenic reactions and possess limited toxicity\(^{[3-5]}\). Consequently, all these properties as well as the ease of surface modification to bear the targetable properties make liposomes more attractive candidates for use as drug-delivery vehicles than other drug carrying systems such as nanoparticles\(^{[6,7]}\) and microemulsions \(^{[8,9]}\). In the 1970s \(^{[10]}\), liposomes were introduced as drug delivery vehicles but the initial clinical results were not satisfactory due to their colloidal and biological instability and their inefficient encapsulation of drug molecules. A schematic representation of liposomal drug delivery is given in Figure 1. Hydrophobic drugs place themselves inside the bilayer of the liposome and hydrophilic drugs are entrapped within the aqueous core or at the bilayer interface. Liposomal formulations enhance the structural and design considerations for liposomal drug delivery.

Fig 1: A schematic representation of liposomal drug delivery
Liposomes can be surface functionalized to endow stealth through PEGylation and to promote receptor-mediated endocytosis by using targeting ligands such as antibodies, peptides, proteins, carbohydrates, and various other small molecules. PEGylation extends liposomal circulation half-life in vivo by reducing clearance, immune recognition, and the non-specific absorption of serum proteins. Polyethylene glycol (PEG) density determines its structure at the liposome surface, with densities below 9% adopting a mushroom-like globular structure and those above 9% adopting a more rigid, extended, brush-like morphology. Chemotherapeutics or diagnostics can be encapsulated into the aqueous lumen, incorporated into the lipid bilayer, or conjugated to the liposome surface. Abbreviation: siRNA, small interfering RNA [11].

METHODS OF PREPARATION:

Loading of drugs in liposome formulations

ENCAPSULATION OF HYDROPHILIC DRUGS

Once lipids are hydrated in the presence of hydrophilic drugs, a portion of the drug gets entrapped inside the liposome and another portion remains in the bulk, outside the aqueous core of the liposome. As only the entrapped drug is of interest, drug in the bulk should be removed. This purification is generally done by gel filtration column chromatography (Sephadex G-50, Pharmacia LKB) and dialysis (hollow fibre dialysis cartridge) on the basis of size differences between the liposomes and the non-encapsulated material. In the cases where DNA or proteins are being encapsulated, or where there is concern that non-encapsulated material may form large aggregates, techniques such as centrifugation can be employed due to the differences in the buoyant densities of liposomes and non-encapsulated material. The pH difference can be brought about by encapsulating a non-permeating buffer ion such as glutamate inside the liposomes at low pH and replacing the extra-liposomal buffer with one which is iso-osmolar at pH 7.0. Alternatively, charged lipids may be incorporated into the membrane at low pH, followed by adjustment of the suspending medium to neutrality. A similar approach may be adopted by using a potassium gradient, in which the membrane is made selectively permeable to potassium ions entrapped inside the liposome by incorporation of valinomycin into the lipid membrane [12, 13].

Encapsulation of hydrophobic drugs

Hydrophobic drugs are solubilized in the phospholipid bilayer of the liposomes that mainly provide a hydrophobic environment. Once trapped, they remain in the lipid bilayer as they have very low affinity towards the inner or outer aqueous regions of the liposomes. During the preparation of liposomes, hydrophobic drugs are solubilized in the organic solvent along with the phospholipids and during the subsequent hydration phase, they remain entrapped in the hydrophobic bilayer region. For example, the liposomal photosensitizer verteporfin (Visudyne) contains a hydrophobic drug that is rapidly transferred to blood proteins in vivo. Activation of the
drug by targeting laser light to blood flowing though the eye causes its site-specific activity in the treatment of wet macular degeneration [14]. Amphotericin B and paclitaxel are the other most commonly investigated hydrophobic drugs in liposome formulations.

STABILITY OF LIPOSOMES
Liposome stability can be explained by physical, chemical and biological means which are all interrelated. Generally, chemical (degradation of phospholipids structures) and physical (uniformity of size distribution and encapsulation efficiency) stability determine the shelf-life of liposomes. Two different types of chemical degradation can affect the performance of the phospholipids bilayers; hydrolysis of the ester bonds linking the fatty acids to the glycerol backbone and oxidation of the unsaturated acyl chains, if present. The level of oxidation can be kept to a minimum by taking some precautions like starting with freshly purified lipids and freshly distilled solvents, avoiding procedures involving high temperatures, carrying out the manufacturing process in the absence of oxygen, deoxygenating the aqueous solutions by passing nitrogen, storing all liposome suspensions in an inert atmosphere and including an anti-oxidant, e.g. α-tocopherol [15]. Hydrolysis type of chemical degradation of the ester linkages in the phospholipid structure occurs most slowly at pH values close to neutral. In general, the rate of hydrolysis has a “V-shaped” dependence, with a minimum at pH 6.5 and an increased rate at both higher and lower pH. In the active loading of drugs, as it is mentioned before, low pH levels are required which triggers the hydrolysis. This hydrolysis kind of chemical degradation is also very effective on the aqueous solutions of liposome due to the presence of water. Temperature also triggers the hydrolysis of the lipids which creates the need for refrigeration. In order to keep hydrolysis to a minimum during active loading, attention must be paid for the removal of residual solvent from the dried lipids. To avoid hydrolysis, instead of ester linked lipids, the usage of ether linkage containing lipids (e.g. found in the membrane of halophilic bacteria) would be an absolute solution [16]. The problems related to the lipid oxidation and hydrolysis during the shelf-life of the liposomal product can be reduced by the storage of liposomal dispersion in the dry state by freeze-drying (lyophilization), without compromising their physical state or encapsulation capacity [17]. However, freeze-drying of liposome systems without appropriate stabilizers will lead to fusion of vesicles, i.e. physical instability. To promote vesicle stability during the freeze-drying process, cycloprotectants [18-20], including saccharides (e.g. sucrose, trehalose, and lactose) and their derivatives are employed [21]. Lyophilization increases the shelf-life of the finished product by preserving in a relatively more stable dry state. Some liposome products on market or clinical trials are provided as lyophilized powder. For example, AmBisomeTM, a liposomal amphotericin, is the first liposome product to be marketed in several countries is supplied as a lyophilized powder to be reconstituted with sterile water injection. Additionally, paclitaxel-liposome
formulations have been developed which show good stability [22]. SUVs have much more tendency to fusion when compared to large liposomes due to the presence of stress arising from the high curvature of the membrane. Since this can occur specifically at the transition temperature of the membrane, it would be better to store these liposomes at a temperature much lower than the transition temperature of the lipids. For example, SUVs should be stored above their transition temperature for no longer than 24 hours but LUVs can be stored for a longer period of time if the temperature of the solution is kept in a range of 4-8 0C for approximately 1 week before the leakage of the encapsulated material starts due to the hydrolytic degradation on the membrane structure.

STERILIZATION OF LIPOSOMES

Pharmaceutical industry in general differentiates between two principally different approaches to ensure sterility of a parental product: terminal sterilization of the final product in its container (steam sterilization) and aseptical manufacturing. Terminal sterilization is the commonly used one because of its higher sterility assurance level achieved when compared with the aseptical methods. However, terminal sterilization is not applicable to many liposomal drug carrier formulations.

Filtration is the most suitable sterilization technique for the thermolabile liposomes since it does not include any form of heat or condition that can result in the degradation of liposomes or leakage of the encapsulated material. However, filtration has some drawbacks such as; being only applicable to the liposomes that are smaller than 200 nm in diameter and being an expensive method due to the equipment requiring to work under high pressure (25 kg/cm² and above). Additionally, this technique must be performed under aseptic conditions [23]. Filtration sterilization is relatively time-consuming and not efficient for the removal of viruses. Studies have shown that polycarbonate membranes are less effective than hydrophobic Fluoropore membrane and cellulose acetate/surfactant-free membrane filtration units [24]. Although the limitations of filtration provoked researches on other sterilization methods, all resulted in the formation of degradation products via the previously mentioned degradation pathways. Filtration and the other methods are summarized according to their applicability on liposomal preparations in Table 1 [25], given above.
### TABLE 1. Summary of the sterilization techniques applied on liposomal preparations

<table>
<thead>
<tr>
<th>Sterilization technique</th>
<th>Advantage(s)</th>
<th>Disadvantage(s)</th>
<th>Convenience</th>
</tr>
</thead>
</table>
| Filtration              | Low operation temperature                                                   | Applicable to liposomes lower than 200 nm in diameter  
Operation under aseptic conditions                                   | Low         |
| γ-irradiation           | Moderate operation temperature  
Highest microbial death reliability                                              | Large scale operation  
Risk of degradation of liposomes                                                            | High        |
| Final steam sterilization | Low cost and convenient                                                     | Risk of degradation of liposomes                                                                   | High        |
| Dry heat                | Low cost and convenient                                                     | Risk of degradation of liposomes                                                                   | High        |
| Ethylene oxide          | Low operation temperature                                                   | Possible carcinogenic residues                                                                    | Low         |
| UV-sterilization        | Low cost and convenient                                                     | Poor penetration into products  
Risk of degradation of liposomes                                                             | High        |
CLINICAL APPLICATIONS OF LIPOSOMES

New drug delivery systems such as liposomes are developed when the existing formulations are not satisfactory. Among all the nanomedicine platforms, liposomes have demonstrated one of the most established nanoplatorms with several FDA-approved formulations for cancer treatment, and had the greatest impact on oncology to date, because of their size, biocompatibility, biodegradability, hydrophobic and hydrophilic character, low toxicity and immunogenicity [26]. Schematic drawing of cytosolic delivery and organelle-specific targeting of drug loaded nanoparticles (i.e. most frequently liposomes) via receptor-mediated endocytosis is shown in Figure 2.

Schematic drawing of the cytosolic delivery and organelle-specific targeting of drug loaded nanoparticles via receptor-mediated endocytosis. After receptor mediated cell association with nanoparticles, the nanoparticles are engulfed in a vesicle known as an early endosome. Nanoparticles formulated with an endosome disrupting property disrupt the endosomes followed by cytoplasmic delivery. On the other hand, if nanoparticles are captured in early endosomes, they may make their way to lysosomes as late endosomes where their degradation takes place. Only fraction of non-degraded drug released in the cytoplasm interacts with cellular organelles in a random fashion. However, cytosolic delivery of a fraction of organelle-targeted nanoparticles via endosomal escape or from lysosomes travel to the targeting organelles to deliver their therapeutic cargo [27].

OCULAR APPLICATIONS

The eye is protected by three highly efficient mechanisms (a) an epithelial layer which is the barrier to penetration (b) tear flow (c) the blinking reflex. All these mechanisms are responsible for the poor drug penetration...
into the deeper layers of the cornea and the aqueous humor and for the rapid wash out of drugs from the corneal surface. Initially, in 1981 the enhanced efficiency of liposomes encapsulated idoxuridine in herpes simplex infected corneal lesions in rabbits was reported [28]. In 1985, it was concluded that ocular delivery of drugs can be either promoted or impeded by the use of liposome carriers, depending on the physicochemical properties of the drugs and the lipid mixture employed [29]. The use of mucoadhesive polymers, carbopol 934P and carbopol 1342 to retain liposomes at the cornea was proposed [30]. While pre-corneal retention times were indeed significantly enhanced under appropriate conditions, liposomes even in the presence of the mucoadhesive had migrated toward the conjunctival sac with very little activity remaining at the corneal surface.

**CANCER THERAPY**

Numerous different liposome formulations of numerous anticancer agents were shown to be less toxic than the free drug. Anthracyclines are drugs which stop the growth of dividing cells by intercalating into the DNA and, thus, kill mainly rapidly dividing cells [18]. These cells are not only in tumors but are also in hair, gastrointestinal mucosa, and blood cells; therefore, this class of drug is very toxic. The most used and studied is Adriamycin. In addition to the above-mentioned acute toxicities, its dosage is limited by its increasing cardio toxicity. Numerous diverse formulations were tried. In most cases, the toxicity was reduced to about 50%. These include both acute and chronic toxicities because liposome encapsulation reduces the delivery of the drug molecules towards those tissues. For the same reason, the efficiency was in many cases compromised due to the reduced bioavailability of the drug, especially if the tumor was not phagocytic or located in the organs of mononuclear phagocytic system. In some cases, such as systemic lymphoma, the effect of liposome encapsulation showed enhanced efficacy due to the continued release effect, i.e., longer presence of therapeutic concentrations in the circulation, while in several other cases, the sequestration of the drug into tissues of mononuclear phagocytic system actually reduced its efficacy [31]. Active targeting of cancer drugs to the tumors is shown schematically in Figure 3.
NEW GENERATION LIPOSOMES

New generation liposomes and their features are summarized in Table 2

<table>
<thead>
<tr>
<th>Type</th>
<th>Main constituent</th>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes</td>
<td>Phospholipids</td>
<td>Excellent biocompatibility due to cellular component</td>
</tr>
<tr>
<td>Archaesomes</td>
<td>One or more lipids containing diether linkages</td>
<td>High stability at several conditions</td>
</tr>
<tr>
<td>Niosomes</td>
<td>Non-ionic surfactant and cholesterol</td>
<td>Less prone to action of bile salts</td>
</tr>
<tr>
<td>Novasomes</td>
<td>Monoester of polyoxyethylene fatty acids, cholesterol and free fatty acids. Two to seven bilayer shells</td>
<td>High loading of drugs</td>
</tr>
<tr>
<td>Transfersomes</td>
<td>Lipid supramolecular aggregates</td>
<td>More flexible hence better transdermal delivery</td>
</tr>
<tr>
<td>Ethosomes</td>
<td>Phospholipids and alcohol in relatively high concentration</td>
<td>More disruptive in the skin lipid bilayer organization hence better transdermal delivery</td>
</tr>
<tr>
<td>Virosomes</td>
<td>Lipids surface modified with fusogenic viral envelope proteins</td>
<td>Intracellular delivery of antigens, drugs and DNA</td>
</tr>
<tr>
<td>Cryptosomes</td>
<td>Phospholipids and polaxamers or PEG</td>
<td>More stable</td>
</tr>
<tr>
<td>Emulsomes</td>
<td>Internal solid fat core surrounded by phospholipid bilayer</td>
<td>Better for encapsulation of hydrophobic drugs</td>
</tr>
<tr>
<td>Vesesomes</td>
<td>Multilamellar liposomes</td>
<td>Multidrug formulations are possible</td>
</tr>
</tbody>
</table>
Type | Main constituent | Advantage
--- | --- | ---
Genosomes | Complex of cationic phospholipids and a functional gene or DNA | Suitable for gene delivery

**CLINICAL APPROVED LIPOSOMES** [32]

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Active Drug</th>
<th>Product Name</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Daunorubicin</td>
<td>Daunoxome</td>
<td>Kaposis sarcoma</td>
</tr>
<tr>
<td>2</td>
<td>Doxurubicin</td>
<td>Mycet</td>
<td>Combinational therapy of recurrent breast cancer</td>
</tr>
<tr>
<td>3</td>
<td>Doxorubicin in PEG-liposomes</td>
<td>Doxil/Caelyx</td>
<td>Refractory Kaposi’s sarcoma; ovarian cancer; recurrent breast cancer</td>
</tr>
<tr>
<td>4</td>
<td>Amphotericin-B</td>
<td>AmBisome</td>
<td>Fungal infections</td>
</tr>
<tr>
<td>5</td>
<td>Cytarabine</td>
<td>DepoCyt</td>
<td>Lymphomatous meningitis</td>
</tr>
<tr>
<td>6</td>
<td>Vincristine</td>
<td>Onco TCS</td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>7</td>
<td>Lurtotecan</td>
<td>NX 211</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td>8</td>
<td>Nystatin</td>
<td>Nyotran</td>
<td>Topical antifungal agent</td>
</tr>
<tr>
<td>9</td>
<td>Al-trans retinoic acid</td>
<td>Altragen</td>
<td>Acute promyelocytic leukemia; renal-cell carcinoma</td>
</tr>
<tr>
<td>10</td>
<td>Platinum compounds</td>
<td>Platar</td>
<td>Solid tumours</td>
</tr>
<tr>
<td>11</td>
<td>Annamycin</td>
<td></td>
<td>Doxorubicin resistant tumours</td>
</tr>
<tr>
<td>12</td>
<td>E1A gene</td>
<td></td>
<td>Various tumours</td>
</tr>
<tr>
<td>13</td>
<td>Liposomes of various drugs and diagnostic agents</td>
<td>Allovectin-7</td>
<td>Broad applications</td>
</tr>
</tbody>
</table>

**CONCLUSION**

From above review I have study the liposomes are better drug delivery system than other. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs. Liposomes can be prepared by disrupting biological membranes (such as by sonication). Liposomal formulations enhance the therapeutic efficiency of drugs in preclinical models and in humans compared to conventional formulations due to the alteration of bio-distribution. Liposome stability can be explained by physical, chemical and biological means which are all interrelated. Generally, chemical (degradation of phospholipids structures) and physical (uniformity of size distribution and encapsulation efficiency) stability
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REFERENCE