Liposomes for Controlled Drug Delivery: Drugs of the Future

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ABSTRACT

The objective of drug delivery systems is to deliver a drug effectively, specifically to the site of action, in a controlled manner and to achieve greater efficacy and minimize the toxic effects compared to conventional drugs. Liposomes are the leading drug delivery systems for the systemic (intravenous) administration of drugs. The real breakthrough developments in the area during the past 15 years have resulted in the approval of several liposomal drugs, and the appearance of many unique biomedical products and technologies involving liposomes. There are now liposomal formulations of conventional drugs that have received clinical approval and many others in clinical trials that bring benefits of reduced toxicity and enhanced efficacy for the treatment of cancer and other life-threatening diseases.

KEYWORDS: Drug delivery system, conventional drugs, liposomal drugs, clinical approval, reduced toxicity

INTRODUCTION

Development of new drug molecule is expensive and time consuming. Improving safety efficacy ratio of “old” drugs has been attempted using different methods such as individualizing drug therapy, dose titration, and therapeutic drug monitoring. Although, the drug delivery system (DDS) concept is not new, great progress has recently been made in the treatment of a variety of diseases. Delivering drug at controlled rate, slow delivery, targeted delivery are other attractive methods and have been pursued vigorously.

Controlled drug delivery technology represents one of the frontier areas of science, which involves multidisciplinary scientific approach, contributing to human health care. These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficacy, reduced toxicity, and improved patient compliance and convenience. Nanocarriers with optimized physicochemical and biological properties are successfully used as delivery tools. Liposomes, solid lipids nanoparticles, dendrimers, polymers, silicon or carbon materials, and magnetic nanoparticles are the examples of nanocarriers that have been tested as drug delivery systems.

Since their discovery in 1965 by Bangham and coworkers, liposomes have attracted considerable interest owing to their organization and the versatility of their physicochemical properties. Due to their versatile nature, liposomes can be used for diverse applications. Liposomal systems became a popular drug delivery platform for several reasons. Liposomes are naturally occurring lipids from living systems, making them nontoxic and biodegradable. The drug is entrapped in liposomes, so it is not exposed to the metabolic machinery of the body, preventing degradation and dilution. Liposomes form spontaneously in aqueous solutions and components can be added or removed in a modular way. As a result of this, their properties can be tailored to the respective application. New advances in liposomal technology are being made to combine drugs or compounds in unique lipids to create stable liposomes. Recently, selective delivery of the anticancer agent doxorubicin in polyethylene glycol (PEG) liposomes for the treatment of solid tumours in patients with breast-carcinoma metastases resulted in a subsequent improvement in survival.

Liposomes

Liposomes are vesicular structures that can form via the accumulation of lipids interacting with one another in an energetically favourable manner. Liposomes were discovered in 1961 by Alec Bangham who was studying phospholipids and blood clotting, and since then they became very versatile tools in biology, biochemistry and medicine. A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane i.e. phospholipids having head group and a tail group. Head is hydrophilic while tail is lipophilic. In the presence of water, the heads are attracted to water and line up to form a surface facing the water. The tails are repelled by water, and line up to form a surface away from the water. In a cell, one layer of head faces outside of the cell, attracted to the water in the environment. Another layer of heads faces inside the cell, attracted by the water inside the cell. The hydrocarbon tails of one layer face the hydrocarbon tails of the other layer, and the combined structure forms a bilayer.

The most common constituent of a liposome is phospholipid that spontaneously forms closed structures in aqueous solution. The phospholipid is a double fatty acid chain, which is primarily respon...
sible for bilayer formation. The diameter of liposomes ranges from 20 nm to several hundreds of nanometers, whereas the thickness of the phospholipid bilayer membrane is approximately 4–7 nm.

**From biomembranes to drug carriers**

First observations of liposomes, meaning fat bodies in Greek, date back in late 19th century where lecithin colloidal solutions were studied. In the early twentieth Century Otto Lehmann observed, what he called artificial cells, which are today rather called multilamellar liposomes. In the mid 60’s Alec Bangham and his colleagues recognized the unique properties of liposomes which are an encapsulation of the part of the solvent into their interior during agitation of the of thin lipid films in water. The potential of liposome-mediated drug delivery to cells was recognized in the 1970s but the mechanisms of liposome-cell interaction have not been elucidated yet completely.

**Classification**

Liposomes can be classified into three groups based on structural parameters: unilamellar vesicles, oligolamellar vesicles and multilamellar vesicles (MLVs). This classification is based on their size and number of bilayers.

In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. Unilamellar vesicles can further be divided into three subclasses:
- Small unilamellar vesicles (SUVs): size ranges from 20-40 nm.
- Medium unilamellar vesicles (MUVs): size ranges from 40-80 nm.
- Large unilamellar vesicles (LUVs): size ranges from 100-1000 nm.

Oligolamellar vesicles are made up of 2-10 bilayers of lipids surrounding a large internal volume.

In multilamellar liposomes, vesicles have an onion structure. Classically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water.

**Evolution of liposomes**

Liposomes have been evolved from a model for biomembranes to drug carriers with clinical utility. The range of medical applications of liposomes extends from chemotherapy of cancer and fungal infections to vaccines and most recently to gene therapy. One of the problems in using liposomes is the fast elimination from the blood and capture of the liposomal preparations by the cells of the reticuloendothelial primarily in the liver. To reduce this problem, number of developments has been made. Table 1 shows the developments in liposomal evolution for long circulation.

**Targeting of liposomes: Passive and active targeting**

Liposomes can be targeted to specific organs or tissues by passive and active methods.

**Passive targeting**

One barrier that limits the application of liposomes is rapid clearance from the blood stream by cells of the mononuclear phagocyte system. The capacity of macrophages can be exploited when liposomes are to be targeted to the macrophages. The introduction of liposomes exhibiting prolonged circulation by virtue of their capability to oppose rapid mononuclear phagocyte system (MPS) uptake represents a milestone in liposomal drug delivery research. Long circulating liposomes spontaneously and selectively accumulate at sites of enhanced vascular permeability that are fortunately present in diseased tissues like tumors and areas of infection and inflammation. This phenomenon is usually referred to as ‘passive targeting’. Passive targeting can differentiate between normal and tumor tissues and has the advantage of direct permeation to tumor tissue.

**Active targeting**

In active targeting, the normal distribution patterns are modified through changes in liposome structure and composition. These changes involve the use of charged lipids or the attachment of a ligand (including proteins, peptides, polysaccharides, glycolipids, glycoproteins and monoclonal antibodies) to deliver the drug to pathologic sites or to cross biological barriers based on molecular recognition processes. In addition, the already mentioned process of liposomal surface coating with PEG is also part of the active targeting strategies, prolonging the half-life of the developed liposome-based formulations. Active targeting allows the increased accumulation of the drug in diseased tissue.

**Table 1: Developmental steps in ‘plain’ liposome evolution**

<table>
<thead>
<tr>
<th>Liposome</th>
<th>Property/Modification</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain liposomes</td>
<td>Simple microscopic vesicles in which an aqueous volume is entirely enclosed by membrane composed of lipid molecules</td>
<td>9</td>
</tr>
<tr>
<td>Immunoliposomes</td>
<td>Immunoglobulins without affecting liposomal integrity or the antibody properties, by covalent binding to the liposome surface or by hydrophobic insertion into the liposomal membrane after modification with hydrophobic residues</td>
<td>14</td>
</tr>
<tr>
<td>Long-circulating liposomes</td>
<td>Coating the liposome surface with inert, biocompatible polymers, such as PEG</td>
<td>15, 16</td>
</tr>
<tr>
<td>Long circulating immunoliposomes</td>
<td>Co-immobilization of an antibody and PEG on the surface of the same liposome</td>
<td>17</td>
</tr>
<tr>
<td>New generation liposomes</td>
<td>Surface can be modified by different methods:</td>
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<tr>
<td>Stealth liposomes</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Transferrin modified liposomes</td>
<td></td>
<td>19</td>
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<tr>
<td>Folate modified liposomes</td>
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<td>20</td>
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Essential factors/quality control assays
The important factors in liposome drug development are drug entrapment efficiency, size, stability, and the ability to scale up the process for manufacturing. Producing liposome-based drugs in large quantities and adhering to the specifications traditionally have been a challenge for pharmaceutical companies. Following quality-control assays should be applied to liposomal formulations 21:

- Basic characterization assays like pH; osmolarity; trapped volume; phospholipid concentration; phospholipid composition; phospholipid acyl chain composition; cholesterol concentration; active compound concentration; residual organic solvents and heavy metals; active compound/phospholipid ratio; proton or ion gradient before and after remote loading.
- Chemical stability assays: phospholipid hydrolysis; non-esterified fatty acid concentration; phospholipid acyl chain auto-oxidation; cholesterol autooxidation; active compound degradation.
- Physical characterization assays: appearance; vesicle size distribution; sub-micron range; micron range; electrical surface potential and surface pH; zeta potential; thermotropic behaviour, phase transition, and phase separation; percentage of free drug.
- Microbiological assays: sterility; pyrogenicity (endotoxin level).

Advantages over conventional dosage
Liposomal drug delivery systems offer several advantages over conventional dosage forms especially for parenteral (i.e. local or systemic injection or infusion), topical, and pulmonary route of administration. The benefits of drug loaded liposomes can be summarized into different categories 12:

- Improved solubility of lipophilic and amphiphilic drugs.
- Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system
- Site-avoidance mechanism: liposomes do not dispose in certain organs, such as heart, kidneys, brain, and nervous system and this reduces cardio-, nephro-, and neuro-toxicity.
- Site specific targeting: in certain cases liposomes with surface attached ligands can bind to target cells (‘key and lock’ mechanism), or can be delivered into the target tissue by local anatomical conditions such as leaky and badly formed blood vessels
- Improved transfer of hydrophilic, charged molecules such as chelators, antibiotics, plasmids, and genes into cells; and
- Improved penetration into tissues, especially in the case of dermally applied liposomal dosage forms.

Drug loading
Drug loading in liposomes can be done either passively or actively. In passive drug loading, drug is loaded during liposome formation while in active loading; drug is loaded after liposome formation. Hydrophobic drugs can be directly combined into liposomes during vesicle formation. Trapping effectiveness is dependent on the solubility of the drug in the liposome membrane 22. Passive encapsulation of water-soluble drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation. Water-soluble drugs that have protonizable amine functions can be actively entrapped by employing pH gradients, which can result in trapping effectiveness approaching 100% 23.

At present, there is no single universal encapsulation method that offers stable encapsulation of most drugs; each drug requires a different approach to manage all of its properties. There have been reports on several approaches to retain more hydrophobic drugs inside liposomes, in the circulation. Most of these methods apply drug precipitation inside preformed liposomes, as low soluble complexes with ions or chemicals. In some cases, drug derivatization is applied to enable active encapsulation of hydrophobic drugs, previously not reported to encapsulate, by active or remote loading 24.

The problem unresolved
The unresolved problem of using liposomal drug delivery systems arises from their accumulation in cells (liver macrophages) outside the target tissues and unpredictable effects dependent on the active agent they carry, such as cellular death 25. In order to overcome this issue, chemical modification of drug carriers with certain synthetic polymers has been frequently employed in an attempt to increase in vivo longevity 26.

Modified liposomes are promising approach for targeted delivery of therapeutics. The multifunctional liposomes, containing the specific proteins, antigens, or other biological substances, can be used to design drugs which act selectively on a particular tissue 2. Liposomes have thus travelled a long journey from ‘plain’ to ‘new generation liposomes’. Table 2 describes the development in liposomal generations.

<table>
<thead>
<tr>
<th>Liposomal generation</th>
<th>Properties</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Conventional liposomes/ First generation liposomes</td>
<td>Liposome-containing amphotericin B, Ambisome, used as an antifungal drug, and doxorubicin-containing liposome</td>
<td>27</td>
</tr>
<tr>
<td>Pure lipid approach/ second generation liposomes</td>
<td>Long-circulating liposomes, such as DaunoXome, a daunorubicin-containing liposome</td>
<td>17</td>
</tr>
<tr>
<td>Surface modified liposomes/ third generation liposomes</td>
<td>Surface-modified liposomes with gangliosides or sialic acid which can evade the immune system responsible for removing liposomes from circulation</td>
<td>28</td>
</tr>
<tr>
<td>Stealth liposomes/fourth generation liposomes</td>
<td>Pegylated liposomal called “stealth liposomes” because of their ability to evade interception by the immune system</td>
<td>18</td>
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</tbody>
</table>
New generation liposomes

Virosomes
A virome is a biological preparation consisting of unilamellar phospholipid membrane (either a mono- or bi-layer) vesicle incorporating virus derived proteins to allow the virosomes to fuse with target cells. Initially, virosomes were intended for the intracellular delivery of drugs and DNA. Later, virosomes became a cornerstone for the development of new vaccines. Special attention has been paid for the studies involving delivery of influenza vaccine using virosomes containing the spike proteins of influenza virus, because it elicits high titres of influenza-specific antibodies. Virosomes can provide an excellent opportunity for the efficient delivery of antigens and many drugs including nucleic acids, cytotoxic drugs and toxoids. The only limitation is they may present certain problems associated with their stability/leakiness and immunogenicity.

Cytoskelton-specific liposomes
Pathological conditions such as hypoxia and inflammation can lead to the development of cell membrane-lesions. An intracellular structure that becomes exposed to the extracellular environment is myosin, a cytoskeletal antigen. Patil et al., 2010 hypothesized that cell viability can be preserved in nascent necrotic cells if the cell membrane lesions were sealed and the injurious conditions removed. Cell membrane lesion sealing and preservation of cell viability were achieved by the application of Cytoskeletal-antigen specific immunoliposomes (CSIL) as molecular “Band-Aid” that initially plugs the holes with subsequent sealing of the lesions. Anti-myosin antibody was chosen as the cytoskeleton-antigen specific antibody to develop CSILs, because antomyosin antibody is highly specific for targeting myosin exposed through myocardial cell membrane lesions in various cardiomyopathies. Cytoskeleton-specific immunoliposomes can fuse with damaged cells, and so they were used as carriers for successful gene delivery into hypoxic cells.

Magnetic liposomes
Liposomes containing magnetite (magnetic liposomes) were first developed in 1981. Various magnetic carriers, including albumin microspheres, erythrocytes and liposomes, have been proposed. Most of these carriers have been administered intra-arterially to obtain highly efficient targeting at a systemic site during the first circulation pass through a strong magnetic field, and the effectiveness of such treatment modalities has been well recognized. The accumulation of these liposomes using an electronic magnet was tested in an animal study. Magnetic liposomes containing doxorubicin were intravenously administered to osteosarcoma-bearing hamsters. When the tumour-implanted limb was placed between two poles of a 0.4 Tesla magnet, the application of the field for 60 minutes resulted in a fourfold increase in drug concentration in the tumour.

CONCLUSION
Liposomes are extremely useful and powerful carrier system for effective and controlled delivery of drugs. Liposomes have moved a long way to become a pharmaceutical carrier of choice for numerous practical applications. The range of medical applications of liposomes extends from chemotherapy of cancer to gene therapy. Liposome technology creates an ample opportunity for formulation and delivery of a wide variety of difficult-to-deliver therapeutic agents, including genes, peptides, siRNA or RNAi, protein, and growth hormones. Currently, liposomal nanoparticles (LNs) encapsulating therapeutic agents, or liposomal nanomedicines, represent an advanced class of drug delivery systems, with several formulations in clinical trials.

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