Clinical application and future direction of liposomal assisted drug delivery system

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Abstract: Liposomes are the small microscopic vesicles in which aqueous volume is entirely enclosed by the membrane composed lipid molecules. Physical characteristics that determine liposome stability in storage and disposition in vivo (in particular, plasma clearance, CL) are some of the most important parameters for parenteral preparations of liposome-based therapeutics. Long-circulating liposomes are now investigated in details and widely used in biomedical in vitro and in vivo studies and have also found their way into clinical practice. The recent development of liposome are the specific binding properties of drug carrying liposome to a target cell, stealth liposomes for targeting hydrophilic drug like doxorubicin, minoxidil, Amphotericin B which leads to reduced toxicity and side effect because the drug is mostly concentrated at site of action. Liposome can also used as adjuvants in vaccines formulation.

Key words: Liposomes, pulmonary adsorption, LET, lipid bilayer, Lipid Vesicles, Phosphidylcholine

INTRODUCTION

Since the discovery in the 1960s that hydration of dry lipid film formed enclosed spherical vesicles or liposomes that resemble miniature cellular organelles with lipid bilayers, the potential use of liposomes as biodegradable or biocompatible drug carriers to increase the potency and reduce the toxicity of therapeutics was recognized. [1] Liposomes are the small microscopic vesicles in which aqueous volume is entirely enclosed by the membrane composed lipid molecules. Liposomes are defined as phospholipid vesicles consisting of one or more concentric lipid bilayer enclosing discrete aqueous spaces. The unique ability of liposomal system to entrap both lipophilic and hydrophilic compound enable of divers range of drug to be encapsulated by these vesicles. Hydrophobic molecules are inserted into the bilayer membrane and hydrophilic molecules can be entrapped in the aqueous center. Liposomal encapsulation technology (LET) is the most up-to-date shipping method utilized by scientific investigators to transmit tablets that act as healing promoters to the assured frame organs. Liposomes form a barrier around their contents, which is proof against enzymes within the mouth and stomach, alkaline solutions, digestive juices, bile salts, and intestinal flora that are generated inside the human frame, as well as free radicals. Only Then Were The first Series Of Liposome-Based Therapeutics Approved For Human Used By The U.S. Food And Drug Administration (FDA). Liposomes Or Lipid Vesicles Are Colloidal Particles That Can Be Prepared With (Phospholipid) Molecules Derived From Either Natural Sources Or Chemical Synthesis. In The 1960s And 1970s, Various Liposome Preparation Methods Were Developed To Study Biological Processes Of Membranes And Membrane-Bound Proteins. By 1970, liposomes were proposed as drug carriers to modify the therapeutic index of a drug by reducing toxicity or increasing efficacy (or both) of the parent drug.

Product	Drug (lipid:drug ratio)	Lipid Formulation	Marketed By	Indication
Doxil™ Caelyx™ DaunoXome™ Ambisome™ Amphotec™ Abelect™	Doxorubicin (8:1) Daunorubicin citrate (15:1) Amphotericin B (3.8:0.4) Amphotericin B (1:1) Amphotericin B (1:1)	PEG-DSPE: HSPC: Cholesterol (5:56:39) DSPC: Cholesterol (2:1) HSPC:DSPG: Cholesterol (2:0.8:1) Cholesteryl sulfate Lipid complex DMPC:DMPG (7:3)	Alza Corporation (formerly, Sequins) Gilead Sciences (formerly, NeXstar) Gilead Sciences (formerly, NeXstar) Alza Corporation (formerly, Sequus) Elan Corporation (formerly, The Liposome Company)	Kaposi sarcoma in AIDS Refractory ovarian cancer Kaposi sarcoma in AIDS Serious fungal infections Cryptococcal meningitis in patients HIV‡ Serious fungal infections

	Serious fungal infections

Table 1 various example of liposome drugs approved for clinical application or under clinical evaluation (in different countries, same drug could be approved for different indications)

Many studies have shown that within the first 15–30 min after intravenous administration of liposomes between 50 and 80% of the dose is adsorbed by the cells of the RES, primarily by the Kupffer cells of the liver.[2,3,4]

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RECENT EXAMPLES OF CONVENTIONAL LIPOSOMES ASSISTED DRUG IN CLINICAL THERAPEUTIC APPLICATIONS

Primary examples are Ambisome® (Gilead Sciences, Foster City, CA, USA) in which the encapsulated drug is the antifungal amphotericin B . Myocet® (Elan Pharmaceuticals Inc., Princeton, NJ, USA) encapsulating the anticancer agent doxorubicin [5], and Daunoxome® (Gilead Sciences), where the entrapped drug is daunorubicin[6] Daunoxome is at present the only pure-lipid MPS-avoiding liposomal formulation; available as a stable ready-to-inject liposomal formulation. Marqibo®, Hana Biosciences, San Francisco, CA), has received orphan medical product designation in US and Europe and has recently been shown to be clinically effective in the treatment of metastatic malignant uveal melanoma depocyt® (Pacira Pharmaceuticals, San Diego, CA) is a slow release liposome-encapsulated cytarabine formulation, recently approved for intrathecal administration in the treatment of neoplastic meningitis and lymphomatous meningitis [7–9]. The Depo-Foam™ platform used in depocyt®, is essentially a spherical 20-mm multi-lamellar matrix comprised of phospholipids/ lipid mixture, similar to normal human cell membranes (phospholipids, triglycerides and cholesterol [10]

Annamycin, a semi-synthetic lipophilic doxorubicin analogue, capable of circumventing multidrug-resistance transporters, was incorporated in tween-containing liposomes. [10-13] A list of FDA-approved liposome formulated drugs and those that are in current clinical trials are presented in Tables 1. The current pharmaceuticals in the United States that are formulated as liposome drug delivery systems are mainly antifungal and anticancer therapies; many more products, including those used as analgesics, gene therapies, and vaccines, are being developed. Because there are a number of recent and excellent review articles on the biophysical aspects of liposome preparation, characterization, and optimization.[14-15]

1. BASIC PROPERTIES OF LIPOSOME:

Liposomes or lipid vesicles are colloidal particles composed of (phospho)lipid molecules as the major constituent in formation of enclosed lipid bilayers or lipid + drug complexes. Although the lipid constituent can vary, many formulations use synthetic products of natural phospholipid, mainly phosphatidylcholinemost of the liposome formulations accepted for human use contain phosphatidylcholine (neutral charge), with fatty acyl chains of varying lengths and degrees of saturation, as a major membrane building block (Table 1). A fraction of cholesterol (30 mol%) is often included in the lipid formulation to modulate rigidity and to reduce seruminduced instability caused by the binding of serum protein to the liposome membrane. Cellular and physiological mechanisms explain the variations of liposome size, charge, surface hydration, membrane fluidity, and clearance of lipidassociated drug. Physical characteristics that determine liposome stability in storage and disposition in vivo (in particular, plasma clearance, CL) are some of the most important parameters for parenteral preparations of liposome-based therapeutics.

Surface charge

Based on the head group composition of the lipid and Phospholipid, liposomes may bear a negative, neutral, or positive charge on their surface. The nature and density of charge on the surface of the liposomes influence stability, kinetics, and extent of biodistribution, as well as interaction with and uptake of liposomes by target cells. Liposomes with a neutral surface charge have a lower tendency to be cleared by cells of the reticuloendothelial system (RES) after systemic administration and the highest tendency to aggregate. Although negatively charged liposomes reduce aggregation and have increased stability in suspension, their nonspeci®c cellular uptake is increased in vivo. Negatively charged liposomes containing phosphatidylserine (PS) or phosphatidylglycerol (PG) were observed to endocytosed at a faster rate and to a greater extent than neutral liposomes.[16,17] Negative surface charge is recognized by receptors found on a variety of cells, including macrophages.[16-18]Inclusion of some glycolipids, such as the ganglioside GM1 or phosphotidylinositol (PI), inhibits uptake mmby macrophages and RES cells and results in longer circulation times. It has been suggested that a small amount of negatively charged lipids stabilize neutral liposomes against an aggregation-dependent uptake mechanism.[15] Positively charged, cationic liposomes, often used as a DNA condensation reagent for intracellular DNA delivery in gene therapy, have a high tendency to interact with serum proteins; this interaction results in enhanced uptake by the RES and eventual clearance by the lung, liver, or spleen. This mechanism of RES clearance partly explains the low in vivo transfection efficiency. Other factors, including DNA instability, immune-mediated clearance, inflammatory response, and tissue accessibility may also contribute to low transfection efficiency in animals. In fact, high doses of positively charged liposomes have been shown to produce varying degrees of tissue inflammation.[19]

Surface hydration

The surface of the liposome membrane can be modified to reduce aggregation and avoid recognition by RES using hydrophilic polymers. This strategy is often referred to as surface hydration or steric modification. Surface modification is often done by incorporating gangliosides, such as GM1 or lipids that are chemically conjugated to hygroscopic or hydrophilic polymers, usually poly(ethyleneglycol) (PEG). This technology is similar to protein PEGylation. Instead of conjugating PEG to therapeutic proteins such as adenosine deaminase (Alderase, for treatment of severe combined immunodeficiency syndrome) to reduce immune recognition and rapid clearance[20]PEG is conjugated to the terminal amine of phosphatidylethanolamine. This added presence of hydrophilic polymers on the liposome membrane surface provides an additional surface hydration layer.[21] The resulting liposomes cannot be recognized by macrophages and RES as foreign particles, and are spared phagocytic clearance. A number of systematic studies have determined the optimum size of PEG polymer and the density of the respective polymeric PEG lipid in the liposome membrane. The optimum size of PEG is MWavg ˆ 1±2000[22] and the presence of PEG± lipid at 5±10 mol% total lipid composition; under these conditions, liposomes can encase both lipophilic and hydrophilic molecules in a stable manner, while avoiding RES uptake. Depending on the length of the PEG polymer, PEG on the liposome membranes occupies an additional 5 nm of surface hydration thickness[23]without Significantly modifying the overall charge property of liposome membranes. One of the key

advantages of using PEG-conjugated lipid is the long-standing human safety data on the use of PEG as excipient for parenteral preparations. However, heterogeneity of long-chain PEG polymers, purified from petroleum products, and the slow renal clearance of extremely large PEG polymers may be concerns.Other amphiphilic polymers with similar properties, such as poly(acryloyl)morpholine (PacM), poly(acrylamide) (PAA), and poly(vinylpyrrolidone) (PVP), have also been conjugated to phospholipids and used as liposome steric protectors with varying degrees of success.[24] Their safety in humans is less well understood.

Fluidity of lipid membrane

Lipid bilayers and liposome membranes exhibit a well-ordered or gel phase below the lipid phase transition temperature (Tc) and a disordered or fluid phase above the Tc. The lipid phase transition is measured and expressed as Tc, the temperature at which equal proportions of the two phases coexist. At a temperature corresponding to Tc, a maximum in liposome leakiness is observed.[25] The phase behavior of a liposome membrane determines permeability, aggregation, protein binding, and, to a lesser degree, fusion of liposomes. Because the Tc varies depending on the length and nature (saturated or unsaturated) of the fatty acid chains the fluidity of bilayers can be controlled by selection and combinations of lipids. For instance, incorporation of cholesterol at a low concentration into the bilayer leads to an increase in the transmembrane permeability, whereas incorporation of higher amounts (> 30 mol%) of cholesterol can eliminate phase transition and decrease the membrane permeability at a temperature >Tc.[26] Various phase transitions of lipid bilayers have been designed to induce liposome fusion and drug release. Encapsulated drugs can be released into the target tissue by modulating local tissue temperature by external heating using various sources of energy, such as infrared, microwave, or laser light. However, drugs bound to lipid membranes or protein-bound lipid membranes may shift the transition temperature or abrogate the phase transition behavior all together.[28,29] Binding of serum proteins also influence the phase transition behavior and release of the aqueous contents of liposomes[30] In addition, fluidity, in particular liposomes that exhibit phase transition behavior at or near physiologic temperatures (37 C), may enhance phospholipase activity at the cell surface, generating lysophospholipids (by deacylation at A1 or A2 positions of phospholipids). Lysophospholipids in rat spinal tissues were shown to produce behavioral neurotoxicity in rats after intrathecal Administration.[31]

Liposome Size

Early research has demonstrated that liposome size affects vesicle distribution and clearance after systemic administration. The rate of liposome uptake by RES increases with the size of the vesicles.[32] Whereas RES uptake in vivo can be saturated at high doses of liposomes or by predosing with large quantities of control liposomes, this strategy may not be practical for human use because of the adverse effects related to the impairment of RES physiological functions. The general trend for liposomes of similar composition is that increasing size results in rapid uptake by RES.[33] Most recent investigations have used unilamellar vesicles, 50±100 nm in size, for systemic drug delivery applications. For example, the antifungal liposome product AmBisome is formulated to the size Specifications of 45±80 nm to reduce RES uptake. Serum protein binding is an important factor that affects liposome size and increases the rate of clearance in vivo. Complement activation by liposomes and opsonization depend on the size of the liposomes.[34,35] Even with the inclusion of PEG in the liposome compositions to reduce serum protein binding to liposomes, the upper size limit of long-circulation PEG±PE liposomes is 150±200 nm. Due to biological constraints, development of long circulating large (> 500 nm) liposomes using steric stabilization methods has not been successful. Hence, considerations of liposome size and its control in manufacturing at an early stage of drug development provides a means to optimize efficiency of liposome drug delivery systems.

Long-circulating Liposomes

One of the drawbacks of the use of liposomes was the fast elimination from the blood and capture of the liposomal preparations by the cells of the RES, primarily, in the liver. To increase liposomal drug accumulation in the desired areas, the use of targeted liposomes with surface-attached ligands capable of recognizing and binding to cells of interest, and potential induction of the liposimal internalization has been suggested (see Fig. 1). Targeted liposomes offer various advantages over individual drugs targeted by means of polymers or antibodies. One of the most compelling advantages is the dramatic increase in drug payload that can be delivered to the target. Furthermore, the number of ligand molecules exposed on the liposome surface can be increased, improving ligand avidity and degree of the uptake. Immunoliposomes also help provide a "bystander kill" effect, because the drug molecules can diffuse into adjoining tumor cells. Immunoglobulins of the IgG class, and their fragments are the most widely used targeting moieties for liposomes (termed "immunoliposomes" after the modification), which Could be attached to liposomes without affecting the liposome integrity and antibody properties by covalent binding to the liposome surface or by hydrophobic insertion into the liposomal membrane after modification with hydrophobic residues [36]
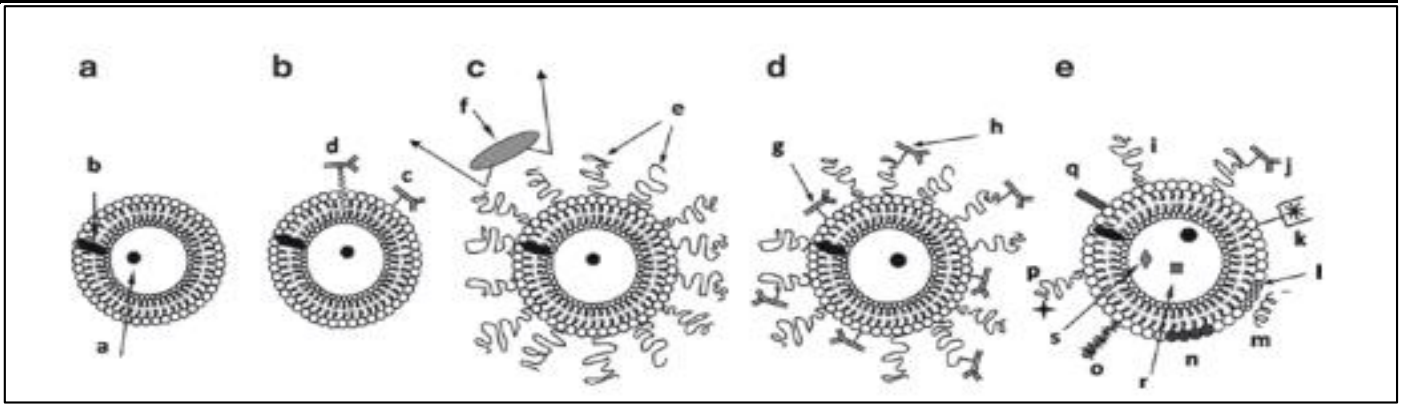
Fig. 1. Evolution of liposomes. (a) Early traditional phospholipids "plain" liposomes with water soluble drug (a) entrapped into the aqueous liposome interior, and water-insoluble drug (b) incorporated into the liposomal membrane (these designations are not repeated on other figures). (b) Antibody-targeted immunoliposome with antibody covalently coupled (c) to the reactive phospholipids in the membrane, or hydrophobically anchored (d) into the liposomal membrane after preliminary modification with a hydrophobic moiety. (c) Long-circulating liposome grafted with a protective polymer € such as PEG, which shields the liposome surface from the interaction with opsonizing proteins (f). (d) Long-circulating immunoliposome simultaneously bearing both protective polymer and antibody, which can be attached to the liposome surface (g) or, preferably, to the distal end of the grafted polymeric chain (h). € New-generation liposome, the surface of which can be modified (separately or simultaneously) by different ways. Among these modifications are: the attachment of protective polymer (i) or protective polymer and targeting ligand, such as antibody (j); the attachment/incorporation of the diagnostic label (k); the incorporation of positively charged lipids (l) allowing for the complexation with DNA (m); the incorporation of stimuli-sensitive lipids (n); the attachment of stimuli-sensitive polymer (o); the attachment of cell-penetrating peptide (p); the incorporation of viral components (q). In addition to a drug, liposome can loaded with magnetic particles ® for magnetic targeting and/or with colloidal gold or silver particles (s) for electron microscopy. Reproduced with permission from (2)

Still, despite improvements in the targeting efficacy, the majority of immunoliposomes ended in the liver as a consequence of insufficient time for the interaction between the target and targeted liposome. Better target accumulation can be expected if liposomes can stay in the circulation long enough, which provides more time for targeted liposomes to interact with the target. Prolonged circulation allows also for liposomes to deliver pharmaceutical agents to targets other than the RES. Different methods have been suggested to achieve long circulation of liposomes in vivo, including coating the liposome surface with inert, biocompatible polymers, such as flexible polyethylene glycol (PEG), which form a steric protective layer over the liposome surface and slows down the liposome recognition by opsonins and subsequent clearance [37,38], see Fig. 1. Moreover, the PEG chains on the liposome surface avoid the vesicle aggregation, improving stability of formulation[39]

It has been shown with a broad variety of examples that, similar to macromolecules, liposomes are capable of accumulating in various pathological areas with affected vasculature (such as tumor, infarcts, and inflammations) via the enhanced permeability and retention (EPR) effect [40,41] and their longer circulations naturally enhances this way of target accumulation. Doxorubicin, incorporated into long-circulating PEGylated liposomes (Doxil®) demonstrates good activity in EPR-based tumor therapy and strongly diminishes the toxic side effects (cardiotoxicity) of the original drug [42]. Evidently, long-circulating liposomes can be easily adapted for the delivery of various pharmaceuticals to tumor and other "leaky" areas. Interestingly, recent evidence showed that PEG-liposomes, previously considered to be biologically inert, still could induce certain side reactions via activation of the complement system [43,44]

In general, PEGylated liposomes demonstrate dose- independent, non-saturable, log-linear kinetics, and increased bioavailability [45], where incorporation of PEG-lipids causes the liposome to remain in the blood circulation for extended periods of time (i.e., $t1/2 > 40$ h) and distribute through an organism relatively evenly with most of the dose remaining in the central compartment (i.e., the blood) and only 10–15% of the dose being delivered to the liver. This is a significant improvement over conventional liposomes where typically 80–90% of the liposome deposits in the liver [45,46,47]

Long-circulating liposomes are now investigated in details and widely used in biomedical in vitro and in vivo studies and have also found their way into clinical practice [42,49]. Although these favorable characteristics have extended clinical applications of PEGylated liposomes, recent research calls for some caution, where more investigation towards multiple dose administration or biodistribution in tumor tissues, is warranted. It was recently reported that intravenous injection in rats of PEG-grafted liposomes may significantly alter the pharmacokinetic behavior of a subsequent dose when this dose is administered after an interval of several days[50]. This phenomenon, called "accelerated blood clearance" (ABC), appears to be inversely related to the PEG content of liposomes. By the same token, an inverse relationship has been observed between dose and magnitude of the ABC effect [48]

Although, PEG remains the gold standard in liposome steric protection, attempts continue to identify other polymers that could be used to prepare long-circulating liposomes. Earlier studies with various water-soluble flexible polymers have been summarized in [51, 52]. More recent papers describe long-circulating liposomes prepared using poly(N-(2-hydroxypropyl)methacrylamide) [53], poly-N-vinylpyrrolidones [54], l-amino acid-based biodegradable polymer-lipid conjugates [55], and polyvinyl alcohol [56].

On the same note, recent research revived the early strategy of liposome surface modification with gangliosides (GM1 and GM3), analogous to erythrocyte membrane, which demonstrated prolonged circulation only in mice and rats. This new application of GM-coated liposomes involved their use for oral administration and delivery to the brain. In particular, Taira et al. [57] suggest that among liposomal formulations used as oral drug carriers, those containing GM1 and GM3 have better possibilities of surviving through the gastrointestinal tract. It was reported that observed higher brain-tracer uptake for GM1 liposomes than for control liposomes in the cortex, basal ganglia, and mesencephalon of both hemispheres; conversely, no significant changes were observed in liposomal liver uptake or blood concentration [58]. Another example includes ovalbumin in PEG-coated liposomes induced the best mucosal immune response of all carriers tested [59]. To improve protein and peptide bioavailability via the oral route, an oral colon-specific drug delivery system for bee venom peptide was developed that was based on coated alginate gel beads entrapped in liposomes[60]

Subcutaneous administration of PEGylated liposomes has shown unique potential especially for targeting to the lymph nodes, achieving sustained drug release in vivo[61] Earlier research by Allen et al. [62] has demonstrated the feasibility of targeting liposomes to the lymph nodes that was explored for lymphatic delivery of methotrexate [63] and for magnetic resonance imaging (MRI) with Gadolinium-loaded liposomes [64].

Attempts have been done to attach PEG to the liposome surface in a removable fashion to facilitate the liposome capture by the cell after PEG-liposomes accumulate in target site via the EPR effect (42) and PEG coating is detached under the action of local pathological conditions (decreased pH in tumors). New detachable PEG conjugates are described in (65), where the detachment process is based on the mild thiolysis of the dithiobenzylurethane linkage between PEG and amino-containing substrate (such as PE). Low pH-degradable PEG-lipid conjugates based on the hydrazone linkage between PEG and lipid have also been described (66, 67).

2. CLINICAL APPLICATIONS OF LONG-CIRCULATING

Liposomes PEGylated liposomal doxorubicin (DOXIL®/Caelyx®) was the first and is still the only stealth liposome formulation to be approved in both USA and Europe for treatment of Kaposi's sarcoma (68) and recurrent ovarian cancer (69, 70). Currently, (DOXIL®/Caelyx®) is undergoing trials for treatment of other malignancies such as multiple myelomas (71), breast cancer (72, 73), and recurrent high-grade glioma (74).

A very similar stealth liposome formulation, but encapsulating cisplatin, SPI-077™ (Alza Corporation, Mountain View, CA, USA), has demonstrated the same evident stealth behavior with an apparent t1/2 of approximately 60–100 h. Phase I/II clinical trials of the drug to treat head and neck cancer and lung cancer (75), were showing promising toxicity profile, yet therapeutic efficacy was lacking (76), mainly due to delayed drug release. Hence, another formulation was evaluated, SPI-077 B103 (Alza Corp., Mountain View, CA, USA); they chose B103, where fully hydrogenated soy PC was replaced by unsaturated phospholipids, to decease rigidity of liposomal membrane, aiming for earlier tendency for cisplatin release. However, released drug was not detected in in vitro systems, plasma, or tumor extracellular fluid after administration of either stealth formulation of liposomal cisplatin (77). Similarly, S-CKD602 (Alza Corp., Mountain View, CA, USA), a PEGylated stealth liposomal formulation of CKD-602 – a semisynthetic analog of camptothecin – was submitted for a Phase I trial. After it was demonstrated that the plasma AUC for S-CKD602 was 50-fold that of non-liposomal CKD-602; and showed minimal toxicity and encouraging therapeutic activity (78).

Lipoplatin™ (Regulon Inc. Mountain View, CA, USA) is another pegylated liposomal cisplatin formulation composed of showed plasma half-life is 60–117 h in clinical study, depending on the dose (Boulikas et al. 2005; Stathopoulos et al. 2005). The study also found that Lipoplatin has no nephrotoxicity up to a dose of 125 mg/m2 every 14 days without the serious side effects of cisplatin. Clinical evaluation of pegylated liposomal formulation of mitoxantrone (Novantrone®, Wyeth Lederle, Madison, NJ, USA), containing cardiolipin, has displayed promising therapeutic results in acute myeloid leukemia, and prostate cancer (79).

APPLICATION OF LIPOSOME:-

The field of liposome research has distanded considerably over last 30 years. It is now possible to engineer a huge range of differing size, phospholipid composition, cholesterol composition, surface morphology suitable for large range of application. Liposome interface with cells in different ways cause liposomal components to be associated with target cell. The liposome carrier can be targeted to liver and spleen and distinction can be made between normal and tumors tissue using tomography. In case of transdermal drug delivery system, liposome has high application. Liposomal drug delivery system when used to target the tumor cell leads to decrease in the toxicity and increase the effectiveness of drug. The targeting of the liposome to the site of action takes place by the attachment of amino acid fragment, such as antibody or protein or proper fragment that target specific receptors cell. Liposomal DNA delivery vector and advance enhancement In the form of LPDI-I and LPD-II are some of the safest and potential most conversant transfer vector which are used to date. DNA vaccination and improved efficiency of gene therapy some of the recent application of liposome. Different modes of drug delivery application have been purposed for the liposomal drug delivery system.

- Increasing drug solublisation (amphotericin B, doxorubicin,minoxidil ,paclitaxels, and cyclosporins)
- Prevention of sensitive drug molecules (cytosine,arabinosa, DNA, RNA, antisense oligo nucleotides, ribozymes)
- Induce intracellular uptake (Anticancer, antiviral and antimicrobial drugs)
- Modified pharmacokinetic and bio-distribution (prolonged or sustained released drug with short circulatory half life.

Several recent development of liposomal drug delivery system with examples:-

1) Liposome for respiratory drug delivery system:-

Liposomes is widely used in different type of respiratory disorders. Liposomal aerosol has multiple advantage over ordinary aerosol. Sustained released, prevention of local irritation, reduced toxicity and improved stability in the large aqueous core.Multiple injectable liposome based product are now in the market including ambisome, fungisome and myocet. To be successfule liposomal drug delivery system for the lung is dependent on following factors :Lipid composition,Size, Charge ,Drug and lipid ratio,Method of drug delivery.The latest use of liposome for the delivery of DNA to the lung mean that a major understanding of their use in macromolecular delivery via inhalation is now emerging. Much of this new knowledge, including new lipid and analytical technique, can be used in the development of liposome based protein formulation. For inhalation of liposome the liquid or dry form is taken and the drug release occurs during nebulization. Drug powder liposome has been produced by milling or by spray drying.
[81,82]

Drug which are formulated in the form of liposome are

Active constituents	Effect
Insulin	Facilitated pulmonary adsorption and increased hypoglycemic effect
Catalase	Conferred resistance to pulmonary oxygen toxicity
Super oxide dusmutase	Minimize toxicity to subsequent hyperoxia and improved survival
Cyclosporins	Preferentially adsorbed by lung and show sustained release
Ricin vaccine	Improved safety profile for intra pulmonary vaccination

Table 2. Drug which are formulated in the form of liposome

2) Liposomes in eye disorder:-

Liposomes has been largely used to treat disorder of both anterior and posterior segment. The disease of eye consist of dry eyes, keratitis, corneal transplant rejection uveitis, endolpthelmitis and proliferative vitro retinopathy. Retinal disease are primary cause of blindness in advanced countries. [83] Liposome is used as vector for genetic transfection and monoclonal antibody directed vehicle. The latest technique of the treatment like applying of focal laser to heat causes release of liposomal drug and dyes are used in the treatment of selective tumor and neo-vescular vessel blocking, angiography, retinal and cloroidal blood vessel stasis. Liposomal drug formulation have been certified for the two of patent drug to date and some other products are under clinical trials. The liposomal drug currently certified are 'verteprofin' for the use in the eye, the profit of the liposome will be applied in treatment, and research aspect of ophthalmology in future. [83]

3) Liposome as immunological adjuvants in vaccines:-

Liposome can encapsulated antigen in their aqueous core or penetrated in the bilayer depending on the lipophilicity of the antigen. Liposome has been demonstrate as immuno- adjuvants, potentiating both cell mediated and non- cell mediated immunity. Liposome increase the immuno response to encapsulated diphtheria toxoid. Liposomes have been used as non toxic adjuvants with bacterial, viral, protozoan, tumor and other antigens. The mechanism by which liposome cause increase in antigens immuno response is not fully understood. Liposomal immuno-adjuvant act by steadily releasing encapsulated antigen on intramuscular injection and also by passively accumulating with in regional lymph node.[84] The aggregation of liposome to lymphoid is done by the targeting of liposome with the help of phospotidyl serine. Liposomal vaccine can be prepared by infusing microbes, soluble antigen, cytokinesis of deoxyribonucleic acid with liposome. The latter stimulating an immune response on expression of antigenic protein. Antigen can be covalently coupled to liposomal membrane. [85] Liposome which are encapsulating antigen are second time encapsulated with in alginate lysine microcapsules, to control antigen release and increase the antibodies responses . liposomal vaccines can be store at the refrigerated condition for about 12 months. Several antigen as liposomal preparation and their application

Antigens as liposomal preparation	Application
Rabies glycoproteins	Interleukin-2 enhance
Cholera toxin .	Enhanced antibody level
Diphtheria toxoid	Superior immuno-adjuvant
Herpes simplex virus	Enhanced antibody level
Hepatitis B virus	Higher antibody response
Bacterial polysaccharide	Superior immuno-adjuvant
Tetanus toxoid	Increase antibody titre
Influenza subunit antigen	Intranasal, protects animal from virus
Carbohydrate antigen	antibody titre in salivary gland

4) Liposomes for brain targeting:

The biocompatible and biodegradable behavior of liposome have recently led to their analysis as drug delivery system to brain.[86] Liposome with a narrow diameter (100nm) as well as broad diameter under go free diffusion through the Blood Brain Barrier (BBB). However it is possible that a small Unilamellar vesicles (SUVS) couple to brain drug transport vectors may be transferred through the BBB by receptor mediated or absorptive mediated transcytosis. Cationic liposome which where invented recently show this structure to under go absorptive mediated endocytosis into cells. Cationic liposome successfully under go absorptive mediated transcytosis through the BBB has not yet been defined. The transport of the substance though BBB by liposomes was extended studied. The important finding issue from there studies are that the addition of the sulphatide (A sulphur ester of galactocerebroside) to liposome composition increases there some latest application ability to cross BBB. Wang et al told that liposomes coated with the mannose rich brain tissue and the mannose coat aid transport of loaded drug through BBB. The neutropeptides , leu –

enekphaline and mefenkephalin kyoforphin commonly do not cross BBB when given systemically. The anti depressant amitriptylline ordinarily penetrate the BBB, due to virtuosity of this method. Nano particles were synthetic with different stabilizer. It was establish that amitriptylline level was significantly increase in brain when the substance was immersed on to the nanoparticle and coated or particle stabilize with polysorbate 85.[86-88]

5) Liposomes as anti infective agent :
Intracellular pathogen like protozoal, bacterial, and fungal reside in the liver and spleen and thus to release this pathogen the therapeutic agent may be targeted to this organ using liposome as a vehicle system. The disease like leishmaniasis , candidiasis aspergillosis, histoplasmosis, erythrococosis , gerardiasis , malaria, and tuberculosis are intended by the respective therapeutic agent using liposome as carrier.

Liposomal preparation for infective disease

Active constituent	Application
Active targeting approach	
Pentamidin	Leishmaniasis
Antisense oligo nucleotides	Leishmaniasis
Anamycin	Leishmaniasis
Asiaticoside	Tuberculosis and leprosy
Rifampicin	Tuberculosis
Passive targeting approach	
Amphotericin B	Meningitis, Leishmaniasis, Candidiasis
Praziquantal	Macrophage activation
Sparfloxacin	M.avium, M. Intracellular IE complex
Gentamycin	Staphylococcal pneumonias

Table 3. Liposomal preparation for infective disease

Use of Amphotericin B, a polyene antibiotic, in the treatment of systemic fungal infection is combine with extensive renal toxicity. Amphotericin B act by the mechanism, in which it binds to sterol in the membrane of sensitive fungi, thus inhancing the membrane permeability. The toxicity of this compound is due to non specificity and mandatory to the mammalian cell cholesterol. Currently the first preparation of Amphotericin B (ambisome) in the form of liposome had passed all clinical trials and now it is used for the treatment of fungal infections. Liposomal Amphotericin B, by passively targeting the liver and spleen, decrease the renal and general toxicity at normal dose but renal toxicity emerged when the drug is given at raised dose due to the saturation of the liver and spleen macrophages. Liposome can also be targeted to lungs by coating vesicle with ostearoyl amylopectin, polyoxylethylene or mono-sialogangliocyte. The encapsulation of antitubercular agent like isoniazid and rifampicin in lung targeted liposome adjust the toxicity and increase the efficacy of these drug. Multiform formulation of the Amphotericin had been confirmed by several clinical trials and are now marketed at different European countries.[89-92]

Different liposomal preparation of Amphotericin B

Preparation	Drug	Targeted site
Liposome (Am Bisome)	Amphotericin B	Systemic fungal infection, visceral leishmaniasis
Liposome (Amphocil)	Amphotericin B	Systemic Systemic fungal infection
Liposome (ABLC)	Amphotericin B	Systemic fungal infection

Table 4. Different liposomal preparation of Amphotericin B

6) Liposome in Intraperitoneal administration
Direct administration of antineoplastic agent into the Intraperitoneal cavity has been suggested to be therapeutically beneficial for cancer that develop in or metastasize to the peritoneal cavity.(93) Intraperitoneal chemotherapy has been a little bit ineffective for free drug because of relatively early clearance of the drug from the Intraperitoneal cavity causing in lowered concentration at the site of action. The clearance of liposomes from the peritoneal cavity is significantly gradual than that of free drug and therefore, higher drug concentration can be achieved in the adjacent of the target side for extended period of time with the use of liposome

formulation. Again reformulation of erosive drug in liposome has been shown to decrease local drug toxicity such as dermal toxicity of doxorubicin. An inhances in TI of paclitaxel in liposome after i.p. administration may also be due to decreasing in local toxicity of the drug. [93-96]

Future Direction

Illumination of physiological liposome disposition mechanism have led to the design of small (~50 nm diameter) and sterically stabilized liposome to increase their systemic resident time required for clinical application. New development in molecular structure for expression of ligand or receptor molecules on the surface of the liposome may enhanced liposome resident time (due to decreased CL) will provide liposome associated drugs a chance to finally reach their intended target sites. A first step to increase the intracellular uptake of liposomal drug (anti-cancer agents, antibiotics, DNA) is to improve it's localization selectively with in the target tissue. As additional ligand with greater affinity and specificity persist to be developed, and improvement is made in antibody engineering to mass produce targeted liposome preparation, liposome-drug complexes with prolonged therapeutic indices are now within reach. Latest, HER2 antibody (binds to erb-2 oncogene product on select tumor cells) imparted on liposome showed good results in preclinical studies. If these results can be proved in human trials, we may soon have targeted liposome delivery system that can potentially be used to formulate high potency drugs with significantly enhanced safety and efficacy. Additional improvement of biomembrane sensors that function effectively (e.g., pH sensitivity for DNA delivery to nuclei) in an in vivo blood and tissue environment will add significantly to the benefits of delivering drug not only to cells, but also to specific organelles with in the target cells, using liposome drug delivery system.

Conclusion:-

Liposome are the small vesicles of spherical structure of lipid bilayer. Encapsulated in the amphiphilic drug and carrying through the cell membrane and BBB. Different parameters are contribute to liposome success as a drug delivery vehicles. Liposome can elongated the drug action and moderately release of drug in the body. Targeting approach modify the distribution of the drug in the body. The recent development of liposome are the specific binding properties of drug carrying liposome to a target cell, stealth liposomes for targeting hydrophilic drug like doxorubicin, minoxidil, Amphotericin B which leads to reduced toxicity and side effect because the drug is mostly concentrated at site of action. Liposome can also used as adjuvants in vaccines formulation. In future for marketing of More advanced and highly stabilized liposomal formulation. In future the liposomal drug delivery system will regenerate the vesicular system with the huge application specifically for in the treatment of

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