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A Novel Approach for Transdermal Drug Delivery as a Liposomes their Progress and Limitations: A Review

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ABSTRACT

The transdermal route of drug delivery has gained great interest of pharmaceutical research, as it circumvents number of problems associated with oral route of drug administration. The uniqueness of this type of drug carrier system lies in the fact that it can accommodate hydrophilic, lipophilic as well as amphiphilic drugs. These drugs find place in different places in the vesicle before they get delivered beneath the skin. Liposomes are micro particulate lipoidal vesicles which are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. Due to new developments in liposome technology, several liposome based drug formulations are currently in clinical trial, and recently some of them have been approved for clinical use. Reformulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through alteration in their bio distribution. This review discusses the potential applications of liposomes in drug delivery with examples of formulations approved for clinical use, and the problems associated with further exploitation of this drug delivery system.

KEYWORDS

Liposome, Amphotericin B, Drug delivery system, Doxorubicin, Pharmacokinetic.

INTRODUCTION

Recent advances in biomedical science and combinatorial chemistry have resulted in the design and synthesis of hundreds of new agents with potential activity against a wide range of therapeutic targets in vitro. However, most of these new drugs fail to live up to their potential in the clinic. For instance, although there are numerous anticancer agents that are highly cytotoxic to tumor cells in vitro, the lack of selective antitumor effect in vivo precludes their use in clinic. One of the major limitations of antineoplastic drugs is their low therapeutic index (TI), i.e. the dose required to produce anti-tumor effect is toxic to normal tissues. The low TI of such drugs may be due to:

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(i) their inability to achieve therapeutic concentrations at the target site (solid tumors); (ii) nonspecific cytotoxicity to critical normal tissues such as bone marrow, renal, GI tract and cardiac tissue; and/or (iii) problems associated with for-mulation of the drug, for example, low solubility in pharmaceutically suitable vehicles, leading to the use of surfactants or organic co-solvents which have their own undesirable side effects. Thus, there is a need for effective delivery systems that not only act as a formulation aid but alter the biodistribution of drugs in such a way that a greater fraction of the dose reaches the target site. Liposomes are micro-particulate or colloidal carriers, usually 0.05-5.0 /~m in diameter which form spontaneously when certain lipids are hydrated in aqueous media[8]. Liposomes are composed of relatively biocompatible and biodegradable material, and they consist of an aqueous volume

entrapped by one or more bilayers of natural and/or synthetic lipids **(Fig. 1).**

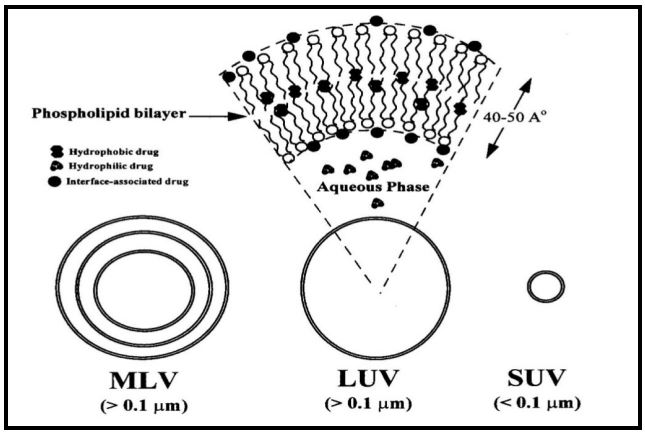

Figure 1: Types of liposomes depending on size and number of lamellae.

Drugs with widely varying lipophilicities can be encapsulated in liposomes, either in the phospholipid bilayer, in the entrapped aqueous volume or at the bilayer interface **(Fig. 1).** Liposomes have been investigated as carriers of various pharmacologically active agents such asanti-neoplastic and antimicrobial drugs, chelating agents, steroids, vaccines and genetic material[29] .Due to recent developments in liposome technology, more effective strategies are now available for controlling the stability and reactivity of liposomes after systemic administration[42] .On the basis of the ability of liposomes to interact with cells and/or blood components, at least two types of liposomes currently can be designed including: (i) non-interactive sterically stabilized (long-circulating) liposomes (LCL) and; (ii) highly interactive cationic liposomes. Sterically stabilized liposomes can be formulated by incorporating hydrophilic long-chain polymers in the bilayer which can form a coat on the liposome surface and repel opsonin penetration and adsorption. Reduction in 'marking' by opsonins leads to slower uptake of these liposomes (LCL) by the cells of reticuloendothelial system (RES). Thus, LCL exhibit extended circulation half-life compared to the so-called 'conventional liposomes' (CL) because of their reduced recognition and uptake by the RES. Furthermore, LCL can be designed to exhibit specific interaction with target cells by attaching target specific ligands. In contrast to LCL, cationic liposomes exhibit high affinity to cell membranes and can be used to deliver exogenous genetic material intracellularly via fusion with the cell membranes[17]. Cationic liposome formulations provide a promising non-viral delivery system for transfection of cells by exogenous plasmids, RNA and oligonucleotides.

Classification of Liposomes

On the Basis of Composition

Liposomes are composed of natural and/or synthetic lipids (phospho- and sphingo-lipids), andmay also contain other bilayer constituents such as cholesterol and hydrophilic polymer conjugated lipids. The net physicochemical properties of the lipids composing the liposomes, such as membrane fluidity, charge density, steric hindrance, and permeability, determine liposomes' interactions with blood components and other tissues after systemic administration. The nature and extent of liposome-cell interaction in turn determines the mode of intracellular delivery of drugs. Thus, the predominant mechanism behind intracellular delivery of drugs by liposomes may mainly depend on their composition, as depicted in **Fig.2.** Liposomes can be classified in terms of composition and mechanism of intracellular delivery into five types as: (i) conventional liposomes (CL); (ii) pH-sensitive liposomes; (iii) cationic liposomes; (iv) immunoliposomes; and (v) long-circulating liposomes (LCL). The typical composition and characteristics for these types of liposomes are listed in **Table 1.**

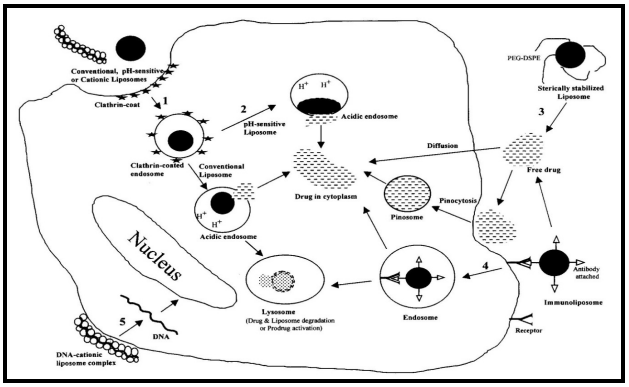

Figure 2: Predominant mechanisms of intracellular drug delivery by liposomes.

The different pathways are indicated by numbers: l-coated pit endocytosis of conventional, pH-sensitive and cationic liposomes; 2-release of drug in the acidic endosome by pH-sensitive liposomes; 3-intravascular and/or extracellular drug release from long circulating liposomes; 4-receptor mediated endocytosis of immunoliposomes; 5-fusion of cationic liposomes with plasma membrane.

On the Basis of Size

The liposome size can range from very small (0.025 /~m) to large (2.5 /~m) vesicles. Furthermore, liposomes may have single or multiple bilayer membranes **(Fig. 1).** The vesicle size is a critical parameter in determining circulation half-life of liposomes, and both size and number of bilayers influence the extent of drug encapsulation in the liposomes. On the basis of their size and number of bilayers, liposomes can also be classified into one of three categories: (i) multilamellar vesicles (MLV); (ii) large unilamellar vesicles (LUV); and (iii) small unilamellar vesicles (SUV). The size and characteristics of these types of liposomes are listed in **Table 2.** The lipids are in a rigid, well-ordered arrangement ('Solid' gel like phase) below the To, and in a liquid-crystalline ('Fluid') phase above the To. The fluidity of liposome bilayers can be altered by using phospholipids with different T~ which in turn can vary from -20 to 90°C depending upon the length and nature (saturated or unsaturated) of the fatty acid chains. Presence of high T-lipids (T c > 37°C) makes the liposome bilayer membrane less fluid at the physiological temperature and less leaky. In contrast, liposomes composed of low T-lipids (T~ < 37°C) are more susceptible to leakage of drugs encapsulated in aqueous phase at physiological temperatures. The fluidity of bilayers may also influence the interaction of liposomes with cells liposomes composed of high Tc lipid appear to have a lower extent of uptake by RES, compared to those containing low Tc lipid[23]. Incorporation of cholesterol into lipid bilayer can also affect the fluidity of the membranes. At high concentrations (> 30 molar %), cholesterol can totally eliminate phase transition and decrease the membrane fluidity at a temperature >Tc, which makes the liposomes more stable and less leaky after systemic administration.

Parameters Which Influence Invivo Behaviour of Liposomes

Liposome formulations of various drugs can be optimized in terms of drug content, stability, desirable bio distribution patterns, and cellular uptake by altering their physicochemical parameters. These parameters include fluidity of bilayer membrane, surface charge density, surface hydration, and size[48]. The effects of these parameters on the physical and biological performance of liposomes are described below.

Bilayer Fluidity

Lipids have a characteristic phase transition temperature (To), and they exist in different physical states above and below the transition temperature (To). The lipids are in a rigid, well-ordered arrangement ('Solid' gel like phase) and in a liquid-crystalline ('Fluid') phase. The fluidity of liposome bilayers can be altered by using phospholipids with different T~ which in turn can vary from -20 to 90°C depending upon the length and nature (saturated or unsaturated) of the fatty acid chains. Presence of high T~ lipids (T c > 37°C) makes the liposome bilayer membrane less fluid at the physiological temperature and less leaky. In contrast, liposomes composed of low T~ lipids (T~ < 37°C) are more susceptible to leakage of drugs encapsulated in aqueous phase at physiological temperatures. The fluidity of bilayers may also influence the interaction of liposomes with cells: liposomes composed of high Tc lipid appear to have a lower extent of uptake by RES, compared to those containing low Tc lipid[23].

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Table 1: Liposome classification based on composition and mode of drug delivery

Type	Composition	Characteristics
Conventional Liposome (CL)	Neutral and/or negatively charged phospholipids pluse Chol.	Subject to coated-pit endocytosis contents ultimately delivered to lysosome, if they do not diffuse from the endosome ,useful for RES targeting rapid and saturable uptake by RES short circulation half-life dose-dependent pharmacokinetics(PK)
pH-sensitive Liposomes	Phospholipid such as PE or DOPE with either CHEMS or OA	Subject to coated-pit endocytosis at low pH fuse with cell or endosome membranes and release their contents in cytoplasm suitable for intracellular delivery of weak bases and macromolecules biodistribution and PK similar to CL
Cationic Liposomes	Cationic lipids, DAB,DOGS, DOSPA, DOTAP, DOTMA, DMRIE and DORIE with DOPE	Possibly fuse with cell or endosome membranes suitable for delivery of negatively charged macromolecule (DNA,RNA,oligos) case of formulation structurally unstable transfection activity decrease with time toxic at high doses mainly restricted to local administration
Long-circulating Liposome (LCL)	Nautral high $T_e$lipids,Chol. Pluse 5-10% of PEG-DSPE,GMI or HPI;≤0.1µm in size	Hydrophilic surface coating low opsonisation and thus low rate of uptake by RES long circulation half-life (-40h) dose independent PK upto 10 µmol/mouse lipid dose.
Immuno-liposome	CL or LCL with attached antibody or recognition sequence	Subject to receptor- mediated endocytosis cell-specific binding (targating) can release contents extracellularly near the target tissue and drug may diffuse through plasma membrane to produce their effects.

Table 2: Liposome classification by size and number of lamellae

Type	Usual size	Characteristics
MLV(Multilamellar vesicles)	>0.1µm	More than one bilayer, moderate aqueous volume to lipid ratio (1-4 L/mole lipid), greater encapsulation of lipophilic drugs, mechanically stable upon long term storage, rapidly cleared by RES, useful for targating the cells of RES, simplest to prepare by thin-film hydration method or hydration of lipid in presence of an organic solvent.
LUV(Large unilamellar vesicles)	>0.1µm	Single bilayer high aqueous volume to lipid ratio (7-1 l/mole lipid) useful for hydrophilic drug high capture of macro-molecules rapidly cleared by RES prepared by detergent dialysis, ether injection, reserve-phase evaporation(REV) or active loading methods.
SUV(Small unilamellar vesicles)	≤0.1µm	Single bilayer homogenous in size, thermodynamically unstable, susceptible to aggregation and fusion at low or no charge, limited capture of macromolecules, low aqueous volume to lipid ratio (0.2-1.5 l/mole lipid) long circulation half-life, prepared by reducing the size of MLV or LUV using probe sonicator or gas extruder or by active loading or solvent injection techniques.

Surface Charge

The nature and density of charge on the liposome surface are important parameters which influence the mechanism and extent of liposome-cell interaction. Both of these parameters can be altered by changing the lipid composition. Lack of surface charge can reduce physical stability of small unilamellar liposomes (SUV) by increasing their aggregation[52]. Moreover, neutral liposomes do not interact significantly with cells, and in such cases, the drug may mainly enter cells after being released from liposomes extracellularly[53]. On the other hand, high electrostatic surface charge could promote liposome-cell interaction. It has been shown that negatively charged liposomes are predominantly taken up by cells through coated-pit endocytosis[54] as depicted in **Fig. 2.**

Studies have also shown that negative charge density influences the extent of liposome interaction with cells[46]. Negative surface charge may not only increase intracellular uptake of liposomes by target cells but by the same mechanism accelerate their plasma clearance after systemic administration[24]. Negatively charged liposomes may also release their contents in the circulation and/or extracellularly after interaction with blood components and tissues. Unlike negatively charged liposomes, cationic liposomes have been proposed to deliver contents to cells by fusion with cell membranes[17].

Surface Hydration

Inclusion of a small fraction (5-10 mole %) of compounds bearing hydrophilic groups such as monosialoganglioside (GM~), hydrogenated phosphatidylinositol (HPI) and polyethylene glycol conjugated with lipid (PEG-DSPE), in the bilayer membrane reduces the interaction of liposomes with cells and blood components and makes liposomes sterically stabilized[2,5,23,51]. As mentioned earlier, the presence of hydrophilic surface coating offers steric hindrance to opsonin adsorption on bilayer which further reduces the rate of liposome uptake by the cells of RES[3]. Thus, the sterically stabilized liposomes are more stable in biological environment and can exhibit up to 10-fold higher circulation half-lives than liposomes without hydrophilic surface coating[39,41].

Liposome Size

Liposome size is one of the main parameters which determine the fraction cleared by the RES[32]. Small liposomes (_< 0.1 /Lm) are opsonized less rapidly and to a lower extent compared to large liposomes (>0.1 /~m) and therefore, the rate of liposome uptake by RES increases with the size of the vesicles. Reduction in liposome size has also been correlated with increased accumulation in tumor tissue. This may partly be due to longer circulation half-life of small liposomes compared to large liposomes. In addition, small liposomes can extravasate by passive convective transport through the tumor capillaries much more easily than large liposomes[46,47]. Tumor capillaries are generally more permeable than normal capillaries and fluid can leak through gaps in the vasculature along with plasma proteins, other macromolecules and micro particulates such as liposomes. Extravasation of liposomes depends on their size and occurs passively; the driving force behind this is the difference between intravascular hydrostatic and interstitial pressures[52,54]. Liposome size can be reduced by sonication, extrusion, or micro fluidization of MLV or LUV.

Method of Preparation

Liposomes of different sizes and characteristics usually require different methods of preparation. The most simple and widely used method for preparation of MLV is the thin-film hydration procedure in which a thin film of lipids is hydrated with an aqueous buffer at a temperature above the transition temperature of lipids. The drug to be encapsulated is included either in the aqueous hydration buffer (for hydrophilic drugs) or in the lipid film (tbr lipophilic drugs). Thin-film hydration method produces a heterogeneous population of MLV (1 5/~m diameter) which can be sonicated or extruded through polycarbonate filters to produce small (up to 0.025 /~m) and more uniformly sized population of SUV. Although

thin-film hydration is a simple technique, one of the major disadvantages of this method is its relatively poor encapsulation efficiency (5 15%) of hydrophilic drugs. Moreover, reduction of liposome size further decreases the amount of encapsulated drug. MLV with high entrapment efficiency (up to 40%) can be prepared by freeze-drying preformed SUV dispersion in an aqueous solution of the drug to be encapsulated[45,48]. The encapsulation efficiency of MLV can also be increased by hydrating lipid in the presence of an organic solvent[25,33,36].

Several methods have been developed for the preparation of large, unilamellar vesicles (LUV), including solvent (ether or ethanol) injection, detergent dialysis, calcium induced fusion, and reverse- phase evaporation (REV) techniques. SUV can be prepared from MLV or LUV by sonication (using probe sonicator) or extrusion (passage through a small orifice under high pressure). In the methods described above, an amphiphilic ionizable drug which exhibits lipophilic and hydrophilic properties depending on the pH of the solution, may not be encapsulated with high efficiency because the drug molecules can diffuse in and out of the lipid membrane. Thus, the drug would be difficult to retain inside liposomes. However, these type of drugs can be encapsulated into preformed liposomes with high efficiency (up to 90%) using the 'active loading' technique[16,18,19]. In the 'active loading' method, the pH in the liposome interior is such that the unionized drug which enters the liposome by passive diffusion is ionized inside the liposome, and ionized drug molecules accumulate in the liposome interior in high concentrations due to their inability to diffuse out through the lipid bilayer. For example, doxorubicin and epirubicin may be entrapped in preformed SUV with high efficacy using 'active loading' methods[41,44,49,50].

Applications of Liposomes in Drug Delivery

New drug delivery systems such as liposomes are developed when an existing formulation is not satisfactory and reformulation offers superior therapeutic efficacy and safety over the existing formulation. Indeed, liposome formulations of some drugs have shown a significant increase in therapeutic efficacy and/or therapeutic indices in preclinical models and in humans, compared to their non-liposomal formulations. The therapeutic applications of liposomes generally fall into several categories briefly described below.

Formulation Aid

Hydrophobic drugs such as cyclosporin and paclitaxel are usually formulated in surfactants and organic co-solvents for systemic administration in humans. These solubilizers may cause toxicity at the doses needed to deliver the drug. In contrast, liposomes are made up of lipids which are relatively non-toxic, non-immunogenic, biocompatible and biodegradable molecules, and can encapsulate a broad range of water-insoluble (lipophilic) drugs. Currently, liposomes or phospholipid mixtures are being used as excipients for preparing better-tolerated preclinical and clinical formulations of several lipophilic, poorly water soluble drugs such as amphotericin B. In preclinical studies, liposomes have been evaluated as a vehicle for the delivery of paclitaxel and its analogs as an alternative to the cremophor/ ethanol vehicle[19,21]. Paclitaxel liposomes were able to deliver the drug systemically and increase the therapeutic index of paclitaxel in human ovarian tumor models[28].

Intracellular Drug Delivery

Drugs with intracellular targets/receptors are required to cross the plasma membrane for pharmacological activity. Liposomes can be used to increase cytosolic delivery of certain drugs such as N-(phosphonacetyl)-L-aspartate (PALA) which are normally poorly taken up into cells[16,17]. PALA is taken up into the tumor cells through fluid-phase endocytosis (pinocytosis) and it diffuses out into the cytoplasm as the endosome pH drops[12]. However, pinocytosis is very limited in its efficiency. Liposomal delivery of drugs which normally enter the cells by pinocytosis can be very effective[35] because liposomes can contain greater concentrations of drug compared to the extracellular fluid and the endocytosis process

by which negatively charged liposomes are predominantly taken up by the cells, is more efficient than pinocytosis. For example, the potency of PALA encapsulated liposomes was up to 500-fold greater against human ovarian tumor cell lines than that of free PALA[32].

Sustained Release Drug Delivery

Sustained release systems are required for drugs such as cytosine arabinoside (Ara-C) that are rapidly cleared in vivo and require plasma concentrations at therapeutic levels for a prolonged period for optimum pharmacological effects[4,9]. It is now possible to design sustained release liposome formulations with an extended circulation half-life and an optimized drug release rate in vivo. For example, Ara-C encapsulated in LCL is effective as a prolonged release system in the treatment of murine L1210/C2 leukemia[3,5]. Conventional liposomes which localize by phagocytosis in the cells of RES may also act as a sustained release depot by slowly leaking drugs from RES into the general circulation.

Gene Therapy[40]

A number of systemic diseases are caused by lack of enzymes/factors which are due to missing or defective genes. In recent years, several attempts have been made to restore gene expression by delivery of the relevant exogenous DNA or genes to cells[8,10]. Cationic liposomes **(Table 1)** have been considered as potential non-viral human gene delivery system[17,19,21,23,28]. They are usually composed of a cationic lipid derivative and a neutral phospholipid (DOPE). The latter is required by certain cationic lipids to form stable liposomes. Some of the widely used cationic liposome formulations are: lipofectin (DOTMA: DOPE, 1:1); lipofectamine (DOSPA: DOPE, 3:1); transfectace (DDAB: DOPE, 1:3); cytofectin(DMRIE: DOPE); transfectam (DOGS) and DC-cholesterol. The negatively charged genetic material (e.g., plasmid) is not encapsulated in liposomes but complexed with cationic lipids, by electrostatic interactions. Plasmid-liposome, complexes are thought to enter the cell by fusion with the plasma or endosome membrane **(Fig. 2).** Allovectin-7, a gene transfer product is currently in clinical trials (phase I/II) as an immune therapeutic agent for the treatment of metastatic melanoma, renal cell and colorectal carcinoma **(Table 3).** Allovectin-7 is composed of a plasmid containing the gene for the major histocompatibility complex antigen HLA-B7 with fl-2 microglobulin formulated with the cytofectin (DMRIE:DOPE). The ongoing clinical trials have indicated that intra-lesional injection ofAllovectin-7 can be performed safely and have demonstrated antitumor activity in some patients[38].

Plasmid-liposome complexes have many advantages as gene transfer vehicles over viral-based vectors[7]:

1. These complexes are relatively nonimmunogenic because they lack proteins;
2. liposomes or lipid complexes can be used for transfection of large-sized genetic material;
3. Viruses, unlike plasmid-liposome complexes, may replicate and cause infections.

Site-Avoidance Delivery

Drugs used in the treatment of diseases like cancer usually have a narrow therapeutic index (TI) and can be highly toxic to normal tissues. The toxicity of these drugs may be minimized by decreasing delivery to critical normal organs. It has been shown that even a small reduction in distribution of the drug to critical organs by encapsulation in liposomes can significantly reduce the drug toxicity[43]. Liposomes are taken up poorly by tissues such as heart, kidney, and GI tract, which are major sites for toxic side-effects of a variety of antineoplastic drugs. Thus, liposome formulation may improve the TI by altering the biodistribution of drug away from drug sensitive normal tissues. Forinstance, free amphotericin B and doxorubicinproduce severe dose-limiting nephrotoxicity and cardiac toxicity, respectively. Reformulation of these

Table 3 Liposome and lipid-based products in clinical trials in USA

Product	Status	Drug	Formulation	Company	Indication/Target
Allovectin-7	HLA-B7 plasmid	DNA-lipid complex(intra lesional injection)	Vical Inc., San Diego, CA	Phase I1 Phase I	Gene therapy of metastatic cancers Gene therapy of metastatic renal cancer with concurrent IL-2
AmBisome^{TM}	AmB(a)	Liposomes	NeXstar Pharmaceutical Inc., Boulder, CO	Pending approval(b)	Life-threatening systemic fungal infection; visceral leishmaniasis
Amphotec^{TM}	AraB	Lipid complex	SequusPharmaceutical,Inc, Menlo Park, CA	Phase III	Life-threatening systemic fungal infection in immune compromised Patients
Annamycin	Annamycin	Liposomes	Aronex Pharmaceuticals, The Woodlands, TX	Phase I/II	Breast cancer
Atragen^{TM}	Tretinoin	Liposomes	AronexPhatmaceuticals, The Woodlands, TX	Phase II/III Phase II Phase I	Kaposi's sarcoma in AIDS. Recurrent acute Promyelocyticleukemia Cancer of blood
Doxil^{TM}	Doxorubicin	Liposomes	Sequus Pharmaceutical Inc., Menlo Park, CA	Phase III	Refractory, ovarian, recurrent breast and prostate cancers
Nyotran^{TM}	Nystatin	Liposomes	Aronex Pharmaceuticals, The Woodlands, TX	Phase II/lI1 Phase I	Candidemia Comparative study against AmB in suspected fungal infection
TLC-D99	Doxorubicin	Liposomes	The Liposome Company, Princeton, NJ	Phase III	Metastatic breast cancer
Ventus ^{TM}	Prostaglandin E1	Liposomes	The Liposome Company, Princeton, NJ	Phase III	Acute Respiratory Distress Syndrome
" Amphotericin B; bNDA filed with United State Food and Drug Administration					

Table 4: Liposome and lipid-based products approved for human use

Product	Drug	Formulation	Company	Indication/target	Country
Doxil™ (a)	Doxorubicin	Liposomes (LCL)	Sequus Pharmaceuticals, inc,CA	Kaposi sarcoma in AIDS	USA and Europe
Ambisome™	Amphotericine B	Liposomes (CL)	NeXstar Pharmaceuticals, inc,Co	Serius fungal infections	Around the world in 24 countries
DaunoXome™	Daunorubicin citrate	Liposomes (LCL)	NeXstar Pharmaceuticals, inc,Co	Kaposi sarcoma in AIDS	USA and Europe
Amphocil™	Amphotericine B	Lipid complex	Sequus Pharmaceuticals, inc,CA	Serius fungal infections	Asia and Europe
Abelcet™	Amphotericine B	Lipid complex	The Liposome Company,NJ	Serius fungal infections	USA and Europe

drugs in liposomes results in reduced toxicity with no change in therapeutic efficacy. Liposome formulations of amphotericin B anddoxorubicin have now been approved for clinical use **(Table 4).**

Site-Specific Targeting

Site-specific delivery, the concept first proposed by Paul Ehrlich[15] involves the delivery of a larger fraction of drug to the target site and therefore, reducing exposure to normal tissues. Liposomes have been employed for accomplishing both passive and active targeting of drugs.

1. Passive Targeting

This approach for liposome drug delivery exploits the natural tendency of certain cells such as Kupffer cells in the liver, and circulating macrophages of RES to phagocytose foreign microparticulates such as liposomes.

Conventional liposome (CL) formulations of drugs and immune stimulators have been successfully used for targeting the cells of RES, and exhibit significant improvement in the TI of the drugs[2,3]. In clinical trials, systemic administration of CL containing muramyl peptide derivatives caused enhancement in the tumoricidal properties of monocytes in patients with recurrent osteosarcoma[17,25,29]. Liposomes have also been used to enhance the antigenicity of certain molecules for new vaccine formulations. Furthermore, CLs have also been employed for targeting of immunosuppressive drugs to lymphatic tissues such as the spleen. In a preclinical model, an increase in immunosuppressive activity, i.e. a delay in heart transplant rejection was observed with CL-encapsulated methylprednisolone[6].

2. Active Targeting

Active targeting of liposome encapsulated drugs may be accomplished by coupling specific antibodies to vesicles (immunoliposomes). Immunoliposomes containing diphtherin toxins (DT) have been shown to provide protection against the non-specific toxicity of DT during cancer chemotherapy [(52)]. Long circulating

immune liposomes (hydrophilic polymer-coated vesicles bound to antibodies and <0.15 /~m in size) can now be designed which may be able to recognize and bind with greater specificity to target cells following systemic administration[27,34,41]. It has been shown that long-circulating immune liposomes (LCI) enhanced therapeutic efficacy of encapsulated doxorubicin in a murine lung tumor model[1]. The effect of size on biodistribution of LCI has been studied in a rabbit model of myocardial infarction[43]. Small sized (0.12-0.15 /Lm) LCI containing infarct-specific antimyosin antibodies (AM) exhibited significantly lower accumulation in RES compared to CL with or without AM. However, the accumulation of LCI-AM was higher in kidneys and lungs compared to CL-AM. The accumulation of large sized (0.35 0.4 /lm) LCI in spleen was 2-fold higher than small sized LCI[48]. Active targeting using immunoliposomes has several advantages over that of antibody-drug conjugates[28]:

(i) immunoliposomes can carry a significantly larger number of drug molecules compared to simple conjugates;

(ii) immunoliposomes can encapsulate drugs with widely varying physicochemical properties; and

(iii) drugs can also reach their intracellular target by diffusion after release from immune liposomes associated with target tissue **(Fig. 2).**

Therefore, unlike antibodies-drug conjugates, in some cases immunoliposomes may not have to undergo receptor mediated-endocytosis to deliver their contents intracellularly.

Intraperitoneai Administration

Direct administration of antineoplastic agents into the intraperitoneal (i.p.) cavity has been proposed to be therapeutically advantageous for cancers that develop in or metastasize to the peritoneal cavity[13]. Intra-peritoneal chemotherapy has been somewhat unsuccessful for free drugs because of relatively fast clearance of the drugs from the i.p. cavity resulting in lowered concentrations at the site of action[33]. However, the clearance of liposomes from the peritoneal cavity is significantly slower than that of free drug and therefore, higher drug concentrations can be achieved in the proximity of the target site for extended periods of time with the use of liposome formulations. Furthermore, reformulation of erosive drugs in liposomes has been shown to reduce local drug toxicity such as dermal toxicity of doxorubicin[18]. An increase in TI of paclitaxel in liposomes after i.p. administration[35] may also be due to a reduction in local (abdominal) toxicity of the drug[8]. The tendency of liposomes to interact with macrophages in RES is exploited in this approach (passive targeting). The mechanism by which liposomes cause increases in antigens' immune response is not fully understood. However, augmentation of liposomal adjuvanticity can be achieved by co-administration of liposome encapsulated antigen with other adjuvants such as lipid A, lipopoly saccharides, muramyldipeptide and interleukin (IL-2)[18,20,23]. Furthermore, antibody-mediated targeting of liposomal to antigen-presenting cells may also improve immune stimulatory effects[3]. The influence of physicochemical properties of the liposomes such as charge density, membrane fluidity and epitope density, on the immune response of the antigen has been extensively studied[27]. For instance, liposome formulations of inactivated encephalomyocarditis and Semliki Forest viruses were significantly more immunogenic when charged phospholipids were used compared to neutral lipids[34,36]. The phase transition temperature (To) of the lipids also appears to influence immunogenicity. For example, immunogenicity of haptens was higher in liposomes composed of lipids with a high Tc than in those with a low T~ [53]. Recently, the first liposome-based vaccine (liposomes containing inactivated hepatitis A virions) was approved for human use in Switzerland and currently, several other liposome-based vaccines are in clinical trials[25].

Limitations of Liposome Technology

As described above, liposomes have a great potential in the area of drug delivery. However,

Liposome-based drug formulations have not entered the market in great numbers so far. Some of the problems limiting the manufacture and development of liposomes have been stability issues, batch to batch reproducibility, sterilization method, low drug entrapment, particle size control, and production of large batch sizes and short circulation half-life of vesicles. Some of these issues such as short half-life have been resolved resulting in increased numbers of clinical trials **(Table 3)** and new approvals **(Table 4).** Some of the remaining problems are discussed in detail below.

Stability[14,37]

One of the major problems limiting the widespread use of liposomes is stability--both physical and chemical. Depending on their composition, the final liposome formulations may have short shelf-lives partly due to chemical and physical instability. Chemical instability may be caused by hydrolysis of ester bond and/or oxidation of unsaturated acyl chains of lipids. Physical instability may be caused by drug leakage from the vesicles and/or aggregation or fusion of vesicles to form larger particles. Both of these processes (drug leakage and change in liposome size) influence the in vivo performance of the drug formulation, and therefore may affect the therapeutic index of the drug. For instance, large liposomes may be rapidly cleared by RES leading to subtherapeutic plasma concentrations of the drug and reduced AUCs (area under the plasma concentration- time curve). Physical instability may also occur due to partitioning out of a hydrophobic drug from the bilayer into the solvent on standing (or long term storage). Some of the stability problems may be overcome by lyophilization in which the final liposome product is freeze-dried with a Cryo-protectant (mostly a sugar like Trehalose) and is reconstituted with vehicle immediately prior to administration. Lyophilization increases the shelf-life of the finished product by preserving it in a relatively more stable dry state. Some liposome products on market or in clinical trials are provided as a lyophilized powder. For example, Ambisome TM, the first liposome product to be marketed in several countries is supplied as a lyophilized powder to be reconstituted with sterile water for injection. Recently, lyophilized paclitaxel- liposome formulations have been developed which show good stability[32].

Encapsulation Efficiency

Liposome formulation of a drug could only be developed if the encapsulation efficiency is such that therapeutic doses could be delivered in a reasonable amount of lipid, since lipids in high doses may be toxic and also cause non-linear (saturable) pharmacokinetics of liposomal drug formulation. Some new approaches that provide high encapsulation efficiencies for hydrophilic drugs have been developed. For instance, active loading of the amphipathic weak acidic or basic drugs in empty liposomes can be used to increase the encapsulation efficiency [(36,39)]. However, active loading is not suitable for hydrophobic drugs such as paclitaxel for which encapsulation efficiency is < 3 mole% mainly due to the low affinity of drug for the lipid bilayers[32].

Active Targeting

One of the major limitations of active targeting using ligand-directed immunoliposomes has been their rapid clearance due to non-specific uptake by the cells of RES. The development of LCL conjugated with ligands has revived interest in this field since LCL are not as rapidly cleared by RES. However, many problems still remain to be overcome. For instance, foreign immunoglobulin-ligands conjugated to immunoliposomes may induce immunogenicity and increase clearance on subsequent exposure[30,31]. The ligand (antibodies) conjugated with liposomes may increase the liposome size and reduce extravasation and thus could limit targeting to intravascular targets[22,26]. It has been shown that size of LCI may be increased in the blood circulation by interaction of the antibodies with serum components, which in turn can increase their size-dependent uptake by spleen[11,12]. Moreover, immune liposome enters the cells by endocytosis **(Fig. 2)** and if

liposome contents are not released from the endosome, they would ultimately be degraded in the lysosomes .This would only be true for drugs sensitive to lysosomal enzymes.

Lysosomal Degradation

Once the liposome has reached the target cell, the efficacy is determined not only by the amount of drug associated with the cell, but also by the amount of drug reaching the 'target molecule' inside the cells. Immunoliposomes may deliver the drug to the cells selectively but the pharmacological activity depends on the ability of intact drug to diffuse into cytoplasm from the endosomes in sufficient amounts.

Liposome Formulations in Market

Liposome and lipid-complex formulations of doxorubicin, daunorubicin and amphotericin B (AmB) have been approved for clinical use in several countries **(Table 4).** Anthracycline glycosides such as doxorubicin and daunorubicin are highly effective antineoplastic drugs; however, they can cause severe cardiac toxicity in humans. For free doxorubicin, the potential for development of irreversible cardiomyopathy is the major dose-limiting factor which restricts the life-time cumulative drug dose in humans to 550 mg/m^2. Long-circulating liposome formulations of anthracyclines have been shown to improve the TI of the drugs against a variety of solid tumors by not only reducing cardiac toxicity but also increasing drug accumulation in tumors[13,15]. Doxorubicin is an ideal candidate for encapsulation in liposomes since it can be encapsulated with high efficiency into liposomes using an active loading method[28]. The first liposome product approved for use in USA was Doxil TM, a LCL formulation of doxorubicin. Doxil TM exhibited up to 8-fold increased circulation half-life compared to free drug[19] and lower incidence of side-effects presumably by avoiding high peak concentrations of the free drug. Encapsulation of doxorubicin in liposomes has increased the TI and made possible dose escalation mainly by reducing the dose-limiting cardiac toxicity. Amphotericin B (AmB) is an antifungal agent used for the treatment of serious systemic fungal infections. However, AmB therapy is associated with high rates of serious side effects which include nephrotoxicity, thrombophlebitis, hypokalemiaand anemia[7,8]. These side effects limit the dose levels which canbe achieved (0.7-1.5 mg/kg of Fungizone TM) and are the major reason for failure or discontinuation of therapy. Liposome- and lipid-based formulations of AraB have been shown to have superior TI over the deoxycholate-based formulation (FungizoneTM) mainly due to a decrease in the dose limiting nephrotoxicity. The incidence of other side-effects is also lower; in some studies these (primarily hypokalemia) ranged from 10 to 20%[16,24]. The reduction in toxicity may be due to selective transfer of AmB from lipid bilayers or complexes to the fungus (target site), thus decreasing the interaction of drug with human cell membranes[22]. The reduction in the extent and frequency of side effects such as nephrotoxicity has allowed for escalation of AmB doses. In summary, this article reviewed the possible applications of liposomes and discussed, in brief, some problems associated with formulation and development. An encouraging sign is the increasing number of clinical trials involving liposome and lipid-based products **(Table 3).** With the newer developments in the field, several companies are actively engaged in expansion and evaluation of liposome products for use in anticancer and antifungal therapy and for prophylaxis (vaccines) against diseases. Further refinements in the liposome technology will spur the full-fledged evolvement of liposomes as drug carriers.

ABBREVIATIONS

AmB--amphotericin B

CL--conventional liposomes

CHEMS--cholesterylhemisuccinate

Chol--cholesterol

DC-chol--3BN (N',N'-dimethylaminoethane)carbamoyl cholesterol

DDAB--dimethyl-dioctadecyl ammonium bromide

DOGS--dioctadecyldimethyl ammonium chloride

DMRIE--1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide

DORIE--1,2-dioleyloxypropyl-3-dimethylhydroxyethylammonium bromide

DOPE--dioleoylphosphatidyl ethanolamine

DOSPA--2,3-dioleoyloxy-N-(2(sperminecarboxamido)-ethyl)- N,N-dimethyl- 1 propa-naminiumfluoroacetate

DOTAP--1,2-dioleoyloxy-3-(trimethylammonio) propane

DOTMA--N-{ 1 -(2,3-dioleoyloxy)propyl}-N,N,N-trimethyl ammonium chloride

FL--fusogenic liposomes

GM1--monosialoganglioside

HPI--hydrogenated phosphatidylinositol

LCI--long-circulating immunoliposomes

LCL--long-circulating liposomes

LUV--large unilamellar vesicles

MLV--multilamellar vesicles

OA--oleic acid

PC--phosphatidylcholine

PE--phosphatidylethanolamine

PG--phosphatidylglycerol

RES--reticuloendothelial system

PEG-DSPE--polyethylene glycol conjugated with distearoyl PE

SUV--small unilamellar vesicles

TI--therapeutic index

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