Liposome- A Versatile Drug Delivery System

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ABSTRACT

Liposomes are microparticulate 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

Key words: Liposomes, hydrogenated phosphatidyl inositol, microparticulates, unilamellar vesicles.Sonication, colloidal carrier.
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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[10]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: (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 formulation of the drug, for example, low solubility in pharmaceutically suitable vehicles, leading to the use of

19

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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 Liposomes are composed of relatively biocompatible and biodegradable material, and they consist of an aqueous volume entrapped by oneor more bilayers of natural and/or synthetic lipids. 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. Liposomes have been investigated as carriers of various pharmacologically active agents such as antineoplastic and antimicrobial drugs, chelating agents, steroids, vaccines and genetic material .Due to recent developments in liposome technology, more effective strategies are now available for controlling the stability and reactivity of liposomes after systemic administration). 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' byopsonins leads to slower uptake of these liposomes(LCL) by the cells of reticuloendothelial. Predominant mechanisms of intracellular drug delivery by liposomes are indicated by 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 extra cellular drug release from long circulating liposomes; 4.receptor mediated endocytosis of immuno liposomes; 5.fusion of cationic liposomes with plasma membrane 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 intra cellularly via fusion with the cell membranes. Cationic liposome formulations provide a promising non-viral delivery system for transfect ion of cells by exogenous plasmids, RNA and oligo nucleotides

Classification of liposomes
A. On the basis of composition
Liposomes are composed of natural and/or synthetic lipids (phospho- and sphingo-lipids), and may 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. 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) immuno liposomes; and (v) long-circulating liposomes (LCL).

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B. On the basis of size

The liposome size can range from very small [7] (0.025 /~m) to large (2.5 /~m) vesicles. Furthermore, liposomes may have single or multiple bilayer membranes. 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) multi lamellar vesicles (MLV) ;(ii) large uni lamellar vesicles (LUV); and (iii) small uni lamellar vesicles (SUV).

Parameters which influence *in vivo* behavior of liposomes

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

a. Bilayer fluidity

Lipids have a characteristic phase transition temperature (To).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 uni lamellar liposomes (SUV) by increasing their aggregation .Moreover, neutral liposomes do not interact significantly with cells, and in such cases, the drug may mainly enter cells after being released from liposomes extra cellularly .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 as depicted in. Studies have also shown that negative charge density influences the extent of liposome interaction with cells. 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. 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

b.Surface hydration

Inclusion of a small fraction (5-10 mole%) of compounds bearing hydrophilic groups such as mono sialo ganglioside (GM~), hydrogenated phosphatidyl inositol(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.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 . 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

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c. Liposome size

Liposome size is one of the main parameters which determines the fraction cleared by the RES. 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. 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 microparticulates such as liposomes. Extra vasation of liposomes depends on their size and occurs passively ;the driving force behind this is the difference between intravascular hydrostatic and interstitial pressures. Liposome size can be reduced by sonication, extrusion, or microfluidization 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 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 (15/~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. More over, 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 . The encapsulation efficiency of MLV can also be increased by hydrating lipid in the presence of an organic solvent .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 wit h 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 (upto 90%) using the 'active loading' technique. 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.

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

22

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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.

1. Formulation aid
Hydrophobic drugs such as cyclosporin and paclitaxel are usually formulated in surfact ants[4] 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. Paclitaxel liposomes were able to deliver the drug systemically and increase the therapeutic index of paclitaxel in human ovarian tumor models

2. Intracellular drug delivery
Drugs with intracellular targets/receptors are required to cross the plasma membrane for pharmacological activity. Liposomes can be used to increase site specific delivery of certain drugs such as N-(phosphonacetyl)-L-aspartate (PALA) which is normally poorly taken up into cells .PALA is taken up in to the tumor cells through fluid-phase endocytosis (pinocytosis) and it diffuses out into the cytoplasm as the endosome However, pinocytosis is very limited in its efficiency. Liposomal delivery of drug which normally enter the cells by pinocytosis can be very effective because liposomes can contain greater concentrations of drug compared to the extracellular fluid and the endcytosis 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 liposome's was up to 500-fold greater against human ovarian tumor cell lines than that of free PALA

3. 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. It is now possible to design sustained release liposome formulations with an extended circulation half-life and an optimized drug release rate *in vivo*. Ara-C encapsulated in LCL is effective as a prolonged release system in the treatment of murine L1210/C2 leukemia .Conventional liposomes which localize by phagocytosis in the cells of RES may also act as sustained release depot by slowly leaking drugs from RES into the general circulation.

4. Gene therapy[6]
A number of systemic diseases are caused by lack of enzymes/factors which are due to missing or defective genes. In recent years, several attempt shave been made to restore gene expression by delivery of the relevant exogenous DNA or genes to cells. Cationic liposomes have been considered as potential non-viral human gene delivery system . They are usually composed of a

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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[16]are: lipofectin; lipofectamine; transfectace: cytofectin; transfectam 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 Allovectin-7, a gene transfer product is currently in clinical trials (phase I/II) as an immunotherapeutic agent for the treatment of metastatic melanoma, renal cell and colorectal .A plasmid containing the gene for the major histo compatibility complex antigen HLA-B7 with fl2microglobulin formulated with the cytofectin(DMRIE:DOPE). The ongoing clinical trials have indicated that intra lesional injection of Allovectin-7 can be performed safely and have demonstrated antitumor activity in some patients .Plasmid-liposome complexes have many advantages as gene transfer vehicles over viral-based vectors (i) these complexes are relatively non immunogenic because they lack proteins; (ii) liposomes or lipid complexes can be used for transfection[13] [14]of large-sized genetic material; and (iii) viruses, unlike plasmid-liposome complexes, may replicate and cause infections. However, there are several problems limiting the application of liposomes as a gene delivery system.

5. 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[16] in liposomes can significantly reduce the drug toxicity. 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 anti neoplastic drugs. Thus, liposome formulation may improve the TI by altering the bio distribution of drug away from drug sensitive normal tissues. Free amphotericin B and doxorubicin produce severe dose-limiting nephrotoxicity and cardiac toxicity, respectively. Reformulation of these drugs in liposomes results in reduced toxicity with no change in therapeutic efficacy. Liposome formulations of amphotericin B and doxorubicin[1] have now been approved for clinical use

6. Site-specific targeting [5] [16] [17]
Site-specific delivery, the concept 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. Liposome and lipid-based products approved for human use. Site specific Liposomal drug delivery of Doxorubicin, Amphotericin B [8], and Daunorubicin are available in the market

7. 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 stimulator shave been successfully used for targeting the cells of RES, and exhibit significant improvement in the TI of the drugs. In clinical trials, systemic administration of CL containing muramyl peptide derivatives caused enhancement in the tumoricidal properties of

24

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monocytes in patients with recurrent osteo sarcoma . 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 methyl prednisolone

8. Active targeting
Active targeting of liposome encapsulated drugs may be accomplished by coupling specific antibodies to vesicles (immune liposomes). Immunoliposomes[12] containing diphtheria toxin (DT) have been shown to provide protection against the non-specific toxicity of DT during cancer chemotherapy. Long circulating immunoliposomes (hydrophilic polymer- coated vesicles bound to antibodies and <0.15 micron in size) can now be designed which may be able to recognize and bind with greater specificity to target cells following systemic administration. It has been shown that long-circulating immunoliposomes[12][13](LCI) enhanced therapeutic efficacy of encapsulated doxorubicin in a murine lung tumor model[18][19].The effect of size on bio distribution of LCI has been studied in a rabbit model of myocardial infarction . Small sized (0.12-0.15 micron) 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 micron) LCI in spleen was 2-fold higher than small sized LCI.Active targeting using immunoliposomes has several advantages over that of antibody-drug conjugates: (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 immunoliposomes associated with target tissue. Therefore, unlike antibodies-drug conjugates, in some cases immunoliposomes may not have to undergo receptor mediated-endocytosis to deliver their contents intracellularly. The limitations of active targeting of drugs using liposomes are discussed in the Section.

9. Intra peritoneaI administration
Direct administration of antineoplastic agents into the intraperitoneal (i.p.) cavity has been proposed to be therapeutically advantageous for cancers. Intraperitoneal 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 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 beeshown to reduce local drug toxicity such as dermal toxicity [17] of doxorubicin An increase in TI of paclitaxel in liposomes after i.p. administration may also be due to a reduction in local (abdominal) toxicity of the drug. . 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 lipopoly saccharides, muramyl dipeptide and interleukin (IL-2) Furthermore, antibody-mediated targeting of liposomal to antigen-presenting

cells may also improve immune stimulatory effects. 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 and . instance liposome formulations of inactivated encephalo myocarditis are significantly more immunogenic when charged phospholipids were used compared to neutral lipids . 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 To than in those with a low To 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

10. Immunological adjuvant in vaccines[2] [3]

Liposomes can encapsulate antigens in their aqueous space or incorporate in the bilayer depending on the lipophilicity of the antigen. Liposomes were first used as immunological adjuvants in order to enhance the immune response to encapsulated diphtheria toxoid.liposome also have been used as nontoxic adjuvants with bacterial, viral, protozoan, tumor and other antigens

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 and new approvals   some of the remaining problems are discussed in detail below.

1. Stability

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 sub therapeutic 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 cryoprotectant (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

2. Sterilization
Identification of a suitable method for sterilization of liposome formulations is a major challenge because phospholipids are thermo labile and sensitive to sterilization procedures involving the use of heat, radiation and/or chemical sterilizing agents. The method available for sterilization of liposome formulations after manufacture is filtration through sterile 0.22 micron membranes. However, filtration is not suitable for large vesicles (>0.2 micron) and also is not able to remove viruses. Sterilization by other approaches such as gamma-irradiation and exposure to chemical sterilizing agents are not recommended because they can cause degradation of liposome components and may leave toxic contaminants Suitable methods for sterilization of liposome formulations are being explored by several groups For instance, it has been shown that under certain conditions, liposomes with thermostable lipophilic drugs could be sterilized by autoclaving without substantial loss of contents and/or degradation of phospholipid

3. 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 amphiphilic[5]weak acidic or basic drugs in empty liposomes can be used to increase the encapsulation efficiency. 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

4. Active targeting
One of the major limitations of active targeting using ligand-directed immuno liposomes 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. The ligand (antibodies) conjugated with liposomes may increase the liposome size and reduce extravasation and thus could limit targeting to intravascular targets 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. Moreover, immunoliposome enter the cells by endocytosis 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

5. Gene therapy[6]
A number of technical problems have to be overcome before cationic liposome-mediated transfection can be fully exploited. For instance, liposomes are significantly less efficient than viral vectors in their trans fection ability. Furthermore, the DNA-lipid complexes are not stable in terms of particle size for long periods of time. In addition, there is lack of *in vivo* targeting

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after systemic administration, and the toxicity of cationic lipids limits the administered dose of the DNA lipid complex. Plasmid-liposomes complexes may be more suited to delivery of genetic material by local administration.

6. 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. 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/ml. 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 . Doxorubicin is an ideal candidate for encapsulation in liposomes since it can be encapsulated with high efficiency into liposomes using an active loading method 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 and a 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, Hypokalemia and anemia. These side effects limit the dose levels which can be 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 formulatio of Ara B have been shown to have superior TI over the deoxycholate-based formulation (Fungizone TM) 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%. 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. 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. 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.

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CONCLUSION

Liposomes are one of the unique drug delivery system, which can be of potential use in controlling and targeting drug delivery. Liposomes are administrated orally, parenterally and topically as well as used in cosmetic and hair technologies, sustained release formulations, diagnostic purpose and as good carriers in gene delivery. One major problem associated in the formulation of liposome due to physicochemical and biological instability. These stability problems can be alleviated by using various methods like lyophilization, proliposome, pH sensitive liposome, microencapsulation and steric stabilization. Nowadays liposomes are used as important carriers for targeted delivery of drug.

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