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Potential of Liposome-incorporated Antimicrobial drugs for treatment in clinically important bacterial strains

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ABSTRACT

This review focuses on the use of liposomes as a drug delivery agent in clinical bacterial strains. Liposomes were preferentially developed because of their composition, which is compatible with biological constituents and unique physicochemical properties, such as ultra small and controllable size, large surface area, high reactivity and functional structure. These properties facilitate the administration of drugs, thereby overcoming some of the limitations in the traditional antibacterial therapeutics. Numerous antibiotics have been prescribed to kill or inhibit the growth of bacteria. Even though the therapeutic efficacy of these drugs is understood, inefficient delivery could result in inadequate therapeutic index, and local and systemic side effects. Research on liposome technology has progressed from conventional vesicles to 'second-generation liposomes', in which long-circulating liposomes can be obtained by modulating the lipid composition, size and charge of the vesicle. It is clear that encapsulation of antibiotics in liposomes has emerged as an innovative and promising alternative that enhances therapeutic efficacy, minimizes undesirable side effects of the drugs, improves the risk-benefit ratio and prevents emerging drug resistant bacteria. The ability and current state of liposomes for delivering various antibiotics are reviewed here, while exploring the shared interests between nano-engineers and microbiologists in developing nanotechnology for the treatment of infectious diseases.

Keywords: Liposomes, Antibiotic carrier, Drug delivery, antibiotic, infectious diseases, bacteria.

INTRODUCTION

An antimicrobial refers to a substance that kills or inhibits the growth of a microorganism. Since the discovery of antibiotics [1], many infectious diseases have been overcome. Antibiotics such as Penicillin are only effective against a narrow range of bacteria, whereas others, like Ampicillin are capable of killing a broad spectrum of gram-positive and negative bacteria [2]. Despite the great progress in antimicrobial development, many infectious diseases remain difficult to cure and treat. One major reason is that many antibiotics are difficult to transport through cell membranes and have low activity inside the cells, thereby imposing negligible inhibition on the intracellular bacteria. In addition, antibiotic toxicity to healthy tissues poses a significant limitation to their use. An alternative approach to the classical delivery of antibacterial therapy resides in associating the drug to a submicroscopic carrier, thereby hiding and protecting the drug from degradation and delivery to inaccessible cells.

Numerous reviews have focused on liposomes being used as drug carriers [3-10]. Liposomes are small vesicles of spherical shape that can be created from cholesterol and natural non–toxic phospholipids. Due to their size, large surface to mass ratio, hydrophilic and hydrophobic characters, liposomes are promising systems for drug delivery [11]. By loading drugs into liposomes through physical encapsulation, adsorption, or chemical conjugation, the

pharmacokinetics and therapeutic index of the drugs can be significantly improved in contrast to the free drug counterparts. The advantages of liposome–based drug delivery includes improving serum solubility of the drug, prolonging the systemic circulation lifetime, releasing drugs at a sustained and controlled manner, preferentially delivering drugs to the tissues and cells of interest, and concurrently delivering multiple therapeutic agents to the same cells for combination therapy [11-13]. The features of liposomes and current management of infectious diseases with an emphasis on the mode of delivery through the use of liposomes will be further discussed here.

LIPOSOME STRUCTURE AND CHARECTERISTICS

G. Gregoriadis and B. Ryman used the property of sequestration of solutes by liposomes to formulate the concept of the liposome drug-carrier [14]. Liposome structure was first described by certain scientists referred [15]. Liposomes are spherical vesicles made up of phospholipids containing a core of aqueous solution [16]. They are able to protect encapsulated therapeutic agents and extend their duration of action, enabling effective intracellular delivery [17-18]. Liposomes are divided from lipids that form a closed bilayer sphere when the hydrophobic phospholipid molecules come into contact with the aqueous environment. This allows the closed sphere to encapsulate water or soluble drugs within the central compartment, while water insoluble drugs can be incorporated to the hydrophobic region of the membrane.

TABLE: 1 CLASSIFICATION OF LIPOSOMES

Vesicle type	Abbreviations	Diameter size	No. of lipid bilayer
Unilamellar vesicle	UV	All size range	One
Small unilamellar vesicle	SUV	20-100nm	One
Medium unilamellar vesicle	MUV	More than 100 nm	One
Large unilamellar vesicle	LUV	More than 100nm	One
Giant unilamellar vesicle	GUV	More than 1 micrometer	One
Oligolamellar vesicle	OLV	0.1-1 micrometer	Approx.1
Multilamellar vesicle	MLV	More than .5 micrometer	25-5
Multivesicular vesicle	MV	More than 1 micrometer	Multi compartmental structure

METHODS OF LIPOSOME PREPARATION

GENERAL METHODS OF PREPARATION

Four basic stages involve in all the methods of preparing the liposomes

1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

METHOD OF LIPOSOME PREPARATION AND DRUG LOADING

Depending on the method of preparation [19-21], liposomes can vary widely in size (0.02-10µm), and in the number of lamellae like, small unilamellar vesicles (SUVs) or oligolamellar (olvs), large unilamellar vesicles (luvs) and multilamellar vesicles (mlvs) depending on their size range.

Table: 2

Preparation method	Vesicle type
Single or oligo-lamellar vesicle made by reverse phase evaporation method	REV
Multi-lamellar vesicle made by reverse phase evaporation method	MLV-REV
Stable pluri-lamellar vesicle	SPLV
Frozen and thawed multi-lamellar vesicle	FAT MLV
Vesicle prepared by extrusion method	VET
Dehydration-Rehydration method	DRV

Methods for generating liposomes include Sonication method [23], e.g.: low sheer rates can result in mlvs and high sheer rates can generate ulvs, extrusion method and heating method [24]. Liposomes form when a sufficient amount of energy (e.g.: via sonication, homogenization, shaking or heating) is supplied to phospholipid placed in water. The most popular and simplest method of mlv preparation is the thin-film hydration procedure [25].

A) Multilamellar Liposomes (MLV)

Lipid Hydration Method: This is the most commonly used method for the preparation of MLV. The method involves drying of a lipid solution so that a thin film is formed at the bottom of the round bottomed flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The procedure is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture .Depending upon their solubility; the drugs to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids. MLV are easy to make by this procedure and a variety of substances can be encapsulated in these liposomes. The negative points of this method are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous. By hydrating the lipids in the presence of an immiscible organic solvent (petroleum ether, diethyl ether), MLVs with high encapsulation efficiency can be prepared. The contents are emulsified by sonication or vigorous vortexing. The organic solvent can be removed by passing nitrogen gas stream over the mixture. Once the organic solvent is removed, MLVs are formed immediately in the aqueous phase. The negative point of this procedure is the exposure of the materials to be encapsulated to sonication and to organic solvent.

Solvent Spherule method for the preparation of mlvs of homogenous size distribution was proposed by Kim et al. (1985). Dispersion in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. When controlled evaporation of organic solvent occurred in a water bath, MLvs were formed.

There are many parameters such as physicochemical characteristics of the liposomal ingredients, materials to be contained within the liposomes, particle size, polydispersity, surface zeta potential, shelf-time, batch–to–batch reproducibility and the possibility for large-scale production of safe and efficient products.

Types of liposomes based on composition and application

Composition of liposomes:

The major structural components of liposomes are Phospholipid and cholesterol. Phospholipid is the major component of the biological membranes. There are two types of phospholipids are used natural (phospatidylcholine) and synthetic phospholipids. It is the amphipathic molecule and also known as lecithin. It is from hens' egg and soya bean. Incorporation of cholesterol in liposome can bring big changes in the preparation by incorporating in to phospholipids membrane in very high concentration up to 1:1 or 2:1 molar ratios of cholesterol to phosphatidylcholine .Since it is an amphipathic molecule ,cholesterol inserts into the membrane with its hydroxyl group oriented towards the aqueous surface and aliphatic chain aligned parallel to the acyl chains in the center of the bilayers and also it increase the separation between choline head groups and eliminates the normal electrostatic and hydrogen bonding interaction. Liposomes that are classified as conventional are negatively charged or neutral, while cationic liposomes impose a positive surface charge. Sterically stabilized long circulating (stealth) liposomes increase circulation time. The targeting agents can be antibodies (immunoliposomes) or other specific ligants (e.g.: peptides) that are attached to the liposome surface either with or without a linker [26].

CONVENTIONAL LIPOSOMES

The first type of liposome is commonly known as conventional liposome, composed of egg phosphatidyl choline and cholesterol. Early work on liposomes as a drug-carrier system used this type of liposomes. They are a family of vesicular structures based on lipid bilayers surrounding aqueous chambers. Conventional liposome's can vary over a great extent in their physicochemical properties such as size, lipid composition, surface charge and number and fluidity of the phospholipids bilayers. By intravenous injection, conventional liposomes are quickly coated with plasma proteins, increasing their phagocytosis by RES cells, and rapidly removed from systemic circulation. Although this has been used in the treatment of parasites that reside in the liver and spleen [26-27], their very short circulating half-life has deterred the initial interest towards conventional liposomes as a delivery vehicle. Modification of liposomal surfaces with protein, peptides, antibodies, carbohydrates and polymers has led to prolonged circulation time [28-29]. To use liposomes for targeting to extra-reticuloendothelial system tissues, a key issue is to reduce the rate of uptake by the RES so as to enable them to remain in the circulation longer. Although derivatives of dicarboxylic acids and dextrans, improve circulation time, the most important breakthroughs in liposome delivery came with uses of the linear synthetic polymer, PEG. The most adapted way to produce long-circulating liposomes is to attach hydrophilic polymer polyethylene glycol (PEG) covalently to the outer surface. Such PEG-coated liposome's are called "sterically stabilized" or stealth liposomes. By chance the most important salient feature of long-circulating liposomes is that they are capable of extravagate at body sites where the permeability of the vascular wall is increased.

Based upon conventional liposomes
1. Stabilize natural lecithin (PC) mixtures
2. Synthetic identical, chain phospholipids
3. Glycolipids containing liposomes

Based upon specialty, the liposomes are classified as
1 - Bipolar fatty acid
2 - Antibody directed liposome.
3 - Methyl/methylene X- linked liposome.
4 - Lipoprotein coated liposome.
5 - Carbohydrate coated liposome.
6 - Multiple encapsulated liposome.

LONG CIRCULATING ''STEALTH'' LIPOSOMES

Hydrophilicity of the liposome can be increased by PEG, resulting in reduced interactions with plasma proteins and lipoproteins [30-32]. Due to steric stabilization accumulation of highly hydrated surface PEG groups that prevent interactions with molecular and cellular biological components [33]. Other polymers include polyacrylamide, polyvinyl alcohol, and polyvinylpyrolidone. These are called as "steric protectors" because of their ability to protect the liposome from elimination by RES. Incorporation of specific glycolipids into liposomes resulted in the avoidance of immediate capture by the MPS cells. These liposomes were named MPS-avoiding liposomes or stealth liposomes. Liposomal pegylation serves two important functions – first, to increase the bioavailability of drugs, and second, it enables slow release of their load so that side effects and toxicity can be reduced [34,35,36]. One of the special features of stealth liposomes is their ability to extravasate at sites where there is high permeability at the vascular walls. Thus the features of the PEG groups are favorable as sites of infection and inflammation have increased capillary permeability.

CATIONIC LIPOSOMES

Cationic liposomes take the place of the youngest member of the liposome family. It is a front-line runner among the delivery systems under development for improving the delivery of genetic material, i.e. useful as a delivery system for genetic material [37, 38, 39, 40, 41]. Their cationic lipid components neutralize negatively charged DNA forming a more compact structure. The resultant DNA-lipid complex provides protection and expression of plasmids and promotes cellular internalization. Cationic liposomes are composed of a positively charged lipid and a co-lipid. Normally used co-lipids include di-oleoyl phosphatidyl ethanol amine (DOPE) or di-oleoyl phosphatidyl choline (DOPC). It is also called helper lipids, in many cases needed for stabilization of liposome complex. One of the most often cited cationic lipids is lipofectin. Commercially a variety of positively charged lipid formulations are available and many are under development. Lipofectin is a commercially available cationic lipid to deliver genes to cells in culture. Lipofectin is a mixture of N-{1-(2, 3-dioleyoyx) propyl-)}-N-N-N-trimethyl ammonia chloride ((DOTMA) and DOPE.

IMMUNO-LIPOSOMES

Immuno-liposomes are able to actively target and recognize specific cells and organs of the body by the presence of antibodies or antibody fragments on the surface of liposomes to enhance target site binding [18]. Factors required for examination are selection of the target antigen, function of the antibody, and type of linker used (e.g. PEG) [79]. The circulation times of liposome's and biodistribution can be influenced by measures such as particle size, lipid composition, surface charge hydration and sensitivity to pH changes, bilayer rigidity, fluidity and the binding kinetics of liposome's to cell surface receptors. Surface –Modified liposomes have been used to improve stability and targeting potential. To prolong the half-life of immune-liposomes after intravenous administration, it can be coated with PEG, thus giving them a greater chance to reach target sites other than MPS macrophages. Although this systems look into for various therapeutic applications, the primary focus has been for targeted delivery of anticancer agents.

CHARACTERIZATION & EVALUATION OF FORMULATION

- Drug-excipients interaction study (FTIR Spectroscopy)
- Field Emission Scanning Electron Microscopic (FESEM) study
- Particle size Distribution Study
- Polydispersity index

- Zeta potential measurements
- Drug loading study
- Lipid quantification and chemical stability
- Level of free drugs
- Liposome stability
- Drug release determination

DESIGNING LIPOSOMES TO ACHIEVE OPTIMIZED PROPERTIES

Drug-loading and control of drug release rate

A very early observation was the difficulty in retaining some types of entrapped molecules in the liposome interior [42, 43, and 44]. Drug release was affected by exposure to serum proteins [45, 46, and 47]. Changing the content of the liposome bilayer, in particular by incorporation of cholesterol [46, 48, and 49] was shown to "tighten" fluid bilayers and reduce the leakage of contents from liposomes. Switching from a fluid phase phospholipid bilayer to a solid phase also reduced leakage [50], as did incorporation of sphingomylin into liposomes [51, 52]. Retention of highly hydrophobic drugs such as paclitaxel in liposomes is problematic [53]. Advance in this part was the development of drug loading in response to transmembrane pH gradients that were generated in response to internal acidic buffers or proton-generating dissociable salts such as ammonium sulfate. This drug loading potential was originally demonstrated for weak bases used to measure pH gradients across membranes, and later was extended to drugs that are weak bases.

Many drugs in current use are weak bases possessing a primary, secondary or tertiary amine that can be loaded in response to pH gradients [54]. Drug retention can be improved by loading drugs to achieve high intra-liposomal drug concentrations above their solubility limits, thus enhancing precipitation or by encapsulating polyanions such as dextran sulfate. The ability of accumulated liposomes to increase the local bioavailable drug concentrations, and increase the therapeutic outcome, only occurs when the rate of release of entrapped drug from the liposomes is optimized.

ANTIMICROBIAL ACTIVITY OF LIPOSOME –ENCAPSULATED DRUGS

The direct effect of antimicrobial agents against pathogens is evaluated by using a disk diffusion method or a micro dilution broth method. Treatment of infections by resistant pathogens is very difficult, and is an important clinical issue. Penicillin-producing organisms such as *Staphylococcus aureus* have caused the virtual elimination of Penicillin from the therapeutic armamentarium against this organism. More recently, *S. aureus* has become resistant to methicillin. The resistance challenge now extends to other gram-negative bacteria such as *Pseudomonas aeruginosa*, and Methicillin-resistant *S. aureus* are known as major refractile organisms of opportunistic infection. However, Nacucchio *et al* ., reported the enhancement of the antibacterial activity of piperacillin against *S. aureus* by liposome encapsulation of the drug [40]. The results expressed as the percentage of bacterial growth inhibition at a 50% MIC of Piperacillin, demonstrated that growth inhibition was the highest when Piperacillin was encapsulated into liposomes. The increased efficacy of liposome-encapsulated Piperacillin or Gentamicin against *P. aeruginosa* and *Escherichia coli* strains resistant to these antibiotics has been reported [55]. Ticarcillin-and tobramycin-resistant strains of *P. aeruginosa* were reported [56], to have a marked increase in sensitivity to antibiotics encapsulated in liposomes. The liposome-encapsulated antibiotics were as effective against the β-lactamase producing strains as against the non-β-lactamase –producing strains.

***In vivo* activity in the treatment of infections:**

Treatment of infections by in vivo techniques includes

- Targeting of β-lactam antibiotics in acute and chronic infections
- Targeting of aminoglycoside antibiotics in acute and chronic infections
- Targeting of the fluoroquinolone antibiotics in acute and chronic infections
- New generations of liposomes for the targeting of non-MPS infected tissues
- Activity *in vitro* on infected cells
- Targeting of β-lactam antibiotics
- Targeting of aminoglycoside antibiotics
- Targeting of the fluoroquinolone antibiotics

PHARMACO-KINETIC CHANGES IN LIPOSOME-ENCAPSULATED DRUGS

Pharmacokinetic data are useful in dosage selection, since *in vitro* assessment of bacterial susceptibility provides an approximate concentration for efficacy. The tissue penetration of antibiotics, that is, the transfer of antibiotics out of the blood, is important, because the drug must leave the blood to cure most infections. The major determinants of the antibiotic tissue concentration are the serum concentration, the level of binding to serum protein, binding at the tissue site, delays in penetration due to membranes, the transport systems that control tissue penetration, blood flow to the tissue site, and the effects of disease on both penetration barriers and local binding sites [57]. Each antibiotic has its own characteristic pharmacokinetic properties, and the application of pharmacokinetic properties. The application of pharmacokinetics in choosing and dosing drugs is one of the practical goals of clinical management of infection.

INTRA-CYTOPLASMIC DELIVERY

Infections are characterized by the ability of the pathogen to remain viable and in some cases, multiply within these phagocytic cells. They include Listeria, Salmonella, Legionella, and Mycobacteria. Organisms contained within these cells are protected from the lethal effects of serum components and extracellular antibiotics. There are 3 ways of drug transportation into cells, these are: passive transport, active transport and pinocytosis.

Macrolides are actively transported into the polymorphonuclear leucocytes via the nucleoside transport system or glycolytic pathway [58, 59]. Macrolides, tetracycline and fluroquinolone antibiotics show high concentrations in the cytoplasm. Aminoglycosides and β-lactam antibiotics show very low penetration. Liposome–encapsulated cephalothin or streptomycin was effective, however, in the intraphagocytic killing of *Salmonella typhimurium* and in experimental salmonellosis [60, 61]. Liposome-encapsulated ampicillin markedly improves the therapeutic activity against listeriosis, due to increased delivery of the drug to macrophages of the liver and spleen [62, 63]. Bakker-woudenberg *et al* ., studied the effect of liposomal encapsulation of Ampicillin on antibacterial activity against intracellular *L. monocytogenes*.

Liposome-encapsulated Amikacin has significantly greater inhibitory activity against the survival of M.avium-intracellular complex inside the peritoneal macrophages than did the free drug [64]. Liposome-encapsulated Amikacin was also effective against the organism in the spleen and kidneys, reducing the colony counts by about 1000-fold when compared with those of both untreated controls and free Amikacin-treated mice [65].

REDUCTION OF DRUG TOXICITY BY LIPOSOMAL INCORPORATION

Aminoglycosides show potent antimicrobial activities against gram-negative bacteria and several types of gram–positive bacteria, but also show nephrotoxicity. They are taken up by the proximal tubular cells of the renal cortex and are sequestered in liposomes, where phospholipase activities were inhibited [66, 67]. Inhibition of phospholipases is partially responsible for aminoglycoside-induced nephrotoxicity and ototoxicity. Encapsulation of aminoglycoside markedly alters its pharmacokinetics and shifts the drugs accumulation from the kidney to other organs, thus reducing nephrotoxicity. Liposome-encapsulated streptomycin was reported to be less acutely toxic than free drug [61]. An 80 mg/kg dose of free streptomycin caused convulsion in mice whereas liposome entrapped dose produced no adverse effects. The toxicity of amphotericin B, which currently limits its clinical usefulness, is caused by its ability to also bind to cholesterol, a component of mammalian cell membrane. Liposomal delivery of amphotericin B represents a unique form of targeting based on the selective transfer of drug from the liposome membrane to the fungal cell membrane, thus minimizing interaction of the drug with the host's cell membranes. Liposome –encapsulated amphotericin B reduced the toxicity of the free drug and allowed higher doses to be administered, thus increasing the therapeutic efficacy of the compound. A single intra-vitreal injection of liposome encapsulated cytosine (cidofovir) was found to have protective and prolonged antiviral effect. The slow rate of release of cidofovir is responsible for the long –term effect.

Drug Loading and Releasing

Loading of drug can be done by two methods:

1 - Incorporation method

2 - Adsorption/Absorption technique.

Release rate of drugs depends on solubility, diffusion and biodegradation of the materials.

TABLE:3 LIPOSOMES FOR ANTIMICROBIAL DRUG DELIVERY

Formulation	Drug	Targeted Microorganism	Activity	References
hydrogenated soy phosphatidylcholine, cholesterol, and distearoylphosphatidylglycerol (DSPG)	Amphotericin B	*Aspergillus fumigates*	targeted drug delivery at infection site	[68]
1,2-dipalmitoyl-sn-glycero-3- phosphocholine (DPPC) and cholesterol	Polymyxin B	*Pseudomonas aeruginosa*	1) decreased bacteria count in lung 2) increased bioavailability 3) decreased lung injury caused by bacteria	[69]
soybean phosphatidylcholine (PC) and cholesterol	Ampicillin	*Salmonella typhimurium*	1) increased stability 2) full biological activity of Ampicillin was observed	[70]
dipalmitoyl-phosphatidylcholine, dipalmitoyl-phosphatidylglycerol, and cholesterol	Ciprofloxacin	*Salmonella dublin*	1) decreased mortality of animals 2) distribution of liposomes to all areas of Infection	[71]
dipalmitoylphosphatidylcholine (DPPC), cholesterol, and dimethylammonium ethane carbamoyl cholesterol (DC-chol)	Benzylpeniciilin	*Staphylococcus aureus*	lower drug concentrations and shorter time of exposure were required	[72]
phosphatidylcholine, cholesterol, and phosphatidylinositol	Netilmicin	*Escherichia coli*	1) reduction in toxicity 2) increased circulation half-life 3) increased survival rate of animal model	[73]
partially hydrogenated egg phosphatidylcholine (PHEPC), cholesterol, and 1,2-distearoylsn-glycero-3-phosphoethanolamine-N-(polyethylene glycol-2000) (PEGDSPE	Gentamicin	*Klebsiella pneumonia*	1) increased survival rate of animal model 2) increased therapeutic efficacy	[74]
hydrogenated soy phosphatidylcholine, cholesterol, and distearoylphosphatidylglycerol (DSPG	Amikacin	Gram negative bacteria	prolonged drug and exposure	[75]
stearylamine (SA) and dicetyl phosphate	Zidovudine	Human immunodeficiency virus	enhanced targeting of ZDV to lymphatic's	[76]
Egg phosphatidyl choline, Diacetyp phosphate and cholesterol,	Vancomycin or teicoplanin	Methicillin - Resistant *Staphylococcus aureus*(MRSA)	1) enhanced each drug uptake by macrophages 2) enhanced intracellular antimicrobial effect of each drug	[77]
Phosphatidyl glycerol, phosphatidyl choline , and cholesterol	Streptomycin	*Mycobacterium avium*	Increased antimicrobial activity	[78]

Various methods can be used for release of the drug:

- Side-by-side diffusion cells with artificial or biological membranes;
- Dialysis bag diffusion technique;
- Reverse dialysis bag technique;
- Agitation followed by ultracentrifugation/centrifugation;
- Ultra-filtration or centrifugal ultra-filtration techniques

Mechanisms of nanoparticle-based antimicrobial drug delivery to microorganisms:

(a) Nanoparticles fuse with microbial cell wall or membrane and release the carried drugs within the cell wall or membrane;

(b) Nanoparticles bind to cell wall and serve as a drug depot to continuously release drug molecules, which will diffuse into the interior of the microorganisms.

ADVANTAGES OF LIPOSOMES

1. Liposomes are biocompatible, flexible, non-toxic, completely biodegradable and nonimmunogenic for systemic and nonsystemic administrations.

2. Potential for delivery of hydrophilic, hydrophobic and amphipathic drugs and agents, liposome's supply both a lipophilic environment and aqueous "milieu interne" in one system.
3. Liposomes have the potential to protect their encapsulated drug from the external environment and to act as sustained release depots(Cyclosporin,Propranolol)
4. Liposomes can be prepared as an aerosol, as a suspension or in a semisolid form such as gel ,cream and lotion, as a dry vesicular powder (prolipsome) for reconstitution or they can be administered through most routes of administration including ocular, pulmonary, nasal, oral, intramuscular ,subcutaneous ,topical and intravenous.
5. Liposomes capable of encapsulating not only small molecules but also macromolecules like superoxide dismutase, haemoglobin, erythropoietin, interleukin-2 and interferon-g.
6. Liposomes has increased stability and reduced toxicity of entrapped drug via encapsulation.(Amphotericin B, Taxol)
7. Liposomes have increased therapeutic index and efficacy of drug (Actinomycin-D).
8. Liposomes help to minimize the exposure of sensitive tissues to toxic drugs.
9. Modify the pharmacokinetic and pharmacodynamic property of drugs(increased circulation life time ,reduced elimination)
10. Potential to couple with site-specific ligands to achieve active targeting (Antimicrobial drugs and anticancer)

CONCLUSION

Liposomes are widely used for intra cytoplasmic pathogen & systemic fungal infection treatment. Liposomes are applied as a drug carrier of antimicrobial agents for treating intra cytoplasmic pathogen infections. Interim most of drug delivery systems that are using liposome now in preclinical process, many have been sanctioned for clinical utility. Drugs loaded in liposome will end up in improved solubility of lipophilic & amphiphilic drugs (such as porphyrins, amphotericin B, minoxidil, some peptides and anthracyclines; hydrophilic drugs, such as doxorubicin or acyclovir, anticancer agent).

Cells get passively targeted, particularly the cells of mononuclear phagocytic system (Antimonials, porphyrins, amphotericin B, vaccines, and immunomodulators). Sustained release of locally or systemically administered drugs is observed in liposomes loaded with Cytosine arabinoside, doxorubicin, cortisones, peptides or biological proteins e.g.: vasopressin. Doxorubicin and amphotericin B are examples of site-avoidance mechanism. Drugs of Anti-inflammatory, anti-infection, anti-cancer are capable of site specific targeting. Liposomes are widely used for delivery of improved transfer-charged molecules, antibiotics, hydrophilic plasmids, chelators, genes, for improved tissue penetration corticosteroids, insulin and anesthetics.

Thus it is explicit that antimicrobial agents that are liposome –encapsulated show improved efficiency against refractory infections compared to other conventional treatment and hence may become successful drugs in the future. Growth in antibacterial therapy will require biochemical and genetics skills as well. These combination carriers could represent more rational design for the improvement of antibacterial therapy.

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