ENHANCED ACTIVITY OF ANTIBIOTICS BY LIPOSOMAL DRUG DELIVERY

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ABSTRACT

Liposome are the most widely used and the most extensively marketed nano-formulation that is being manufactured by pharmaceutical industries. Liposome can be modified in different size and structure. Conjugation of ligend with liposome surface increase the target specificity and changes the pharmacokinetic distribution of encapsulated drug. Different methods of preparation can produce different types of liposomes. Many marketed formulations are available as liposome and has proved to be more useful than the conventional formulations. Antibiotics of different classes such as quinolones, aminoglycosides, beta-lactams, cephalosporins, retroviral, macrolides and polypeptides are associated with the shortcomings of drug toxicities, lower bioavailability as well as bacterial resistance. A proper drug delivery system can circumvent these drawbacks. The liposome can prove to be a big stride towards abolishment of these drawbacks. The disadvantage associated with this novel delivery system should also be understood and prevented by means of proper scientific methods for a betterment of human health and society.

KEYWORDS:

Liposome, Antibiotics, Pharmacokinetics, Targeted drug delivery, Drug toxicity.

1.INTRODUCTION

The name liposome is the combination of two Greek words: 'Lipos' = fat and 'Soma' = body. Structurally, liposomes are concentric vessels in which an aqueous volume forms a core surrounded by a membranous lipid bilayer. The membranes are composed of phospholipids, which are molecules that have a hydrophilic head group and a hydrophobic tail group. The hydrophilic head is attracted towards water, and the hydrophobic tail, is repelled by water [1].

Upon disruption of membrane phospholipids, they tend to rearrange itself into tiny vesicles, either as bilayers or monolayers. The bilayer structures results in "*liposomes*" whereas monolayer structures results in "*micelles*".

Due to unique properties, liposomes can be used for drug delivery. A liposome is a vessel of aqueous core surrounded by hydrophobic membrane which prevents dissolved hydrophilic solutes to pass through the lipids. Hydrophobic chemicals are dissolved into the membrane, and so liposome can be used for the delivery of both hydrophobic molecules and hydrophilic molecules.

For delivery of the molecules to a targeted site, the lipid bilayer fuses with other bilayers for example cell membrane, thus delivering the liposomal contents. These are used for the delivery of a large number of drugs[12].Another strategy for liposomal drug delivery is by the targeting of endocytosis events. The construction of liposome can be done in different size range that makes them liable moieties for purpose of natural macrophage phagocytosis. The liposomes can then be digested while in the macrophage's phagosome, which eventually releases its drug.

Liposomes can also be incorporated with opsonins and ligands for site-specific drug delivery system. A unique property of liposome is to target cancerous cells in body. The endothelial wall of human blood vessels is surrounded by endothelial cells which are tightly packed with each other. Such a tight packing can stop large particles in the blood from leaking. A tumour area does not have close packing between the cells and hence are leaky. This is called as the Enhanced Permeability and Retention effect. Some of the liposome in size less than 400 nm rapidly enter tumour sites from the blood and release the drug.

Liposomes are also relatively non-toxic and biodegradable which results in wider range of biomedical applications.

Advantages

The liposomal drug delivery system offers following advantages[1].

Passive targeting to tumor tissues

The hydrophobic anti-cancer drugs can be incorporated on lipid membrane of liposome that will increase the solubility of poorly soluble anti-cancer drugs. Moreover binding of PEG and site-specific ligands helps in the targeting for anti-neoplastic drugs. This will result in increased effectiveness of drug and decreased toxic side-effects of anti-cancer medicines[2].

Increased potency and efficacy

The liposomes are available in Nano-size range (50-150nm) that makes it permeable to the most of biological membranes of the body. This results in higher accumulation of drug at the targeted site of action. So, more drugs become available for binding to the receptor site. This eventually increases the therapeutic index and efficacy of drug[3].

Higher Stability

The potent drug remains packed under the hydrocarbon chain packing of lipid molecules. This prevents the enzymatic degradation of the drug and provides retention of drug in the circulation. Cholesterol introduced in the formulation makes the phosphatidylcholine more ordered and stable. Thus, the stability of the drug is increased by the mode of drug encapsulation[4].

Decreased toxicity

The effective dose required for treatment of the disease is reduced due to higher availability of dose. This helps in reduced dosing and decreased toxicity. The amount of dose taken decreases and this will decrease the presence of drug inside the body. This eventually results in decrease side-effects and adverse events occurring due to high dose[5].

Site avoidance effect

The anti-cancer drug kills both the cancerous cells as well as normal cells which results in various side-effects. Liposome gets poorly accumulated in soft tissues such as kidney and heart so; destructive effect of anti-neoplastic agents is not caused on this tissues. These results in a site-avoidance effect[6].

Better Pharmacokinetics

PEG coated liposome which is also called as Stealth liposome has longer circulation time in systemic circulation. This provides better availability of the drug for a longer period of time. Moreover, liposomes have been shown to have reduced elimination half-lives. This results in reduced administration of drug and an increase in the therapeutic index of the drug.

Targeted drug delivery

A targeted drug delivery can be achieved with liposome by attaching different site-specific ligands on the surface of liposomal membrane. Due to attachment of such site-specific ligands, the drug produces its effects at the desired site only and reduces the probabilities of drug related toxicities[7].

Protection against enzyme degradation of drugs

Liposome protects the entrapped drug against enzymatic degradation while the drug is in circulation. Lipids used in their formulation are less susceptible to enzymatic degradation. Due to these, the entrapped drug is protected inside the lipid vesicles which keep on circulating in the extracellular fluid.

Drug targeting

The targeting of drugs by mode of liposome is achieved by means of site-specific ligands like apoproteins, sugar residues, hormones or antibodies that can be tagged on the lipid vesicles. The ligand helps in recognising the specific receptor sites and, these results in concentration of lipid vesicles at such target sites. Following this approach the otherwise distribution of liposome into the reticuloendothelial system (RES) is restricted or minimized.

Topical drug delivery

Liposomal formulations have also been applied on skin surface for effective delivery of drugs through the skin. Liposome has a unique property of increasing the skin permeability for the encapsulated drugs and it also diminishes the side effect of these drugs because of reduced dosage forms.

Treatment of HIV infected patients

Several new drugs such as antiretroviral nucleotide have been developed nowadays for curing patients suffering of HIV infections. These encompass antisense oligonucleotide, a novel antiviral agent that has been helpful in treatment of HIV infection. Liposomes can serve as vehicle for delivery of such oligonucleotides and can become a boon for AIDS patients[13].

Enhanced antimicrobial efficacy/safety

An antimicrobial agent needs to be encapsulated in liposomes because of two advantages. One it protects the encapsulated drug from enzymatic hydrolysis. As an illustration, the penicillins and cephalosporin are sensitive to the degradative action of J-lactamase that is generated by some infective agents. The second being, lipid nature of these formulation, it can easily permeate cell membrane of micro-organisms and increase cellular concentration, that eventually reduces the required dose and toxicity as found in the case of liposomal formulation of amphotericin B[14, 15].

Structural components of liposome

The main components of liposomes are

I. Phospholipids
II. Cholesterol

Phospholipids

Phospholipids are major structural components of biological membrane such as cell membrane. The most common phospholipid used is phosphatidylcholine (PC). The molecules of PC are insoluble in water. Phosphatidylcholine can be obtained from both natural as well as synthetic sources. Natural phospholipids are phosphatidylcholine, phosphatidylethanolamine (PE) and phosphatidylserine (PS) whereas synthetic phospholipids include Dioleoylphosphatidylcholine (DOPC), Disteroylphosphatidylcholine (DSPC) and Dioleoylphosphatidylethanolamine (DOPE).

Cholesterol

The introduction of cholesterol in liposome bilayer brings major changes in preparation of membranes. Cholesterol do not form component of bilayer structure but used in formulation as fluidity buffer i.e. below the phase transition temperature, it makes the membrane of increased permeability and above phase transition temperature, it makes the membrane of higher stability. Cholesterol can be incorporated in phospholipid membrane in concentration of 1:1 or 1:2 molar ratios of cholesterol to PC.

Classification of liposome

The liposome can be classified in different ways based on the structure, formulation process and applications[8].

I. Based on size and structure
II. Based on formulation process
III. Based on composition and applications

2. BASED ON SIZE AND STRUCTURE

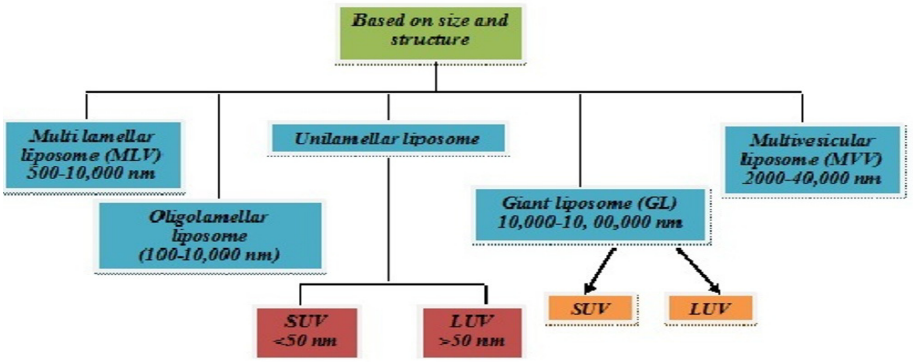

Fig.1 Classification of liposome based on size and structure

a.Multilamellar liposome (MLV)

These liposome ranges in different size from 500-10,000 nm. It is composed of more than one lamella. This liposome can be prepared by lipid film hydration and solvent spherule method.

b.Oligolamellar liposome

The oligolamellar liposome contains less layers of lamella as compared to multilamellar liposome. Their size ranges from 100-10,000 nm in size.

c,Unilamellar liposome

The single lamellar liposome can be called as Unilamellar liposome. They can be further divided as small Unilamellar liposome (SUV) and large Unilamellar liposome (LUV). These can be obtained by different methods like sonication, French pressure cell and other size reducing techniques.

I. Small Unilamellar liposome (SUV) - These are of extremely small size i.e. less than 50 nm.

II. Large Unilamellar liposome (LUV) - These are liposome of larger size i.e. more than 50 nm.

d.Giant liposome (GL)

These are the largest size liposome ranging in size of 10,000-10, 00,000nm. This giant liposome can be used for different diagnostic and medical purposes. They can be both SUV as well as LUV.

e.Multi vesicular liposome (MVV)

In these liposome, drug is encapsulated in two vesicles, first in one vessel and secondly by lipid vesicle. These can be multifunctional liposome ranging in size from 2,000-40,000 nm[(9)].

3.BASED ON THE FORMULATION PROCESS

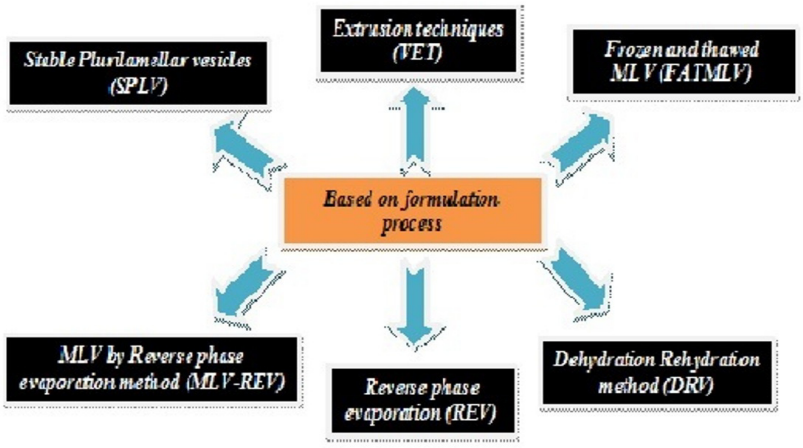

Fig.2 Classification of liposome based on formulation process

The parameters that influence method of preparation liposome preparation are as follows:

a) Physical and chemical nature of drug to be entrapped and liposomal ingredients.
b) Nature of the solvent in which the lipid vesicles are dispersed.
c) Concentration of the entrapped drug and its potential side-effects.
d) Process involved in delivery of vesicles.
e) Optimum size, polydispersity and shelf-life of the vesicles for the required effects.
f) Batch to batch reproducibility of lipoidal formulations[10, 11].

C.Based on composition and applications

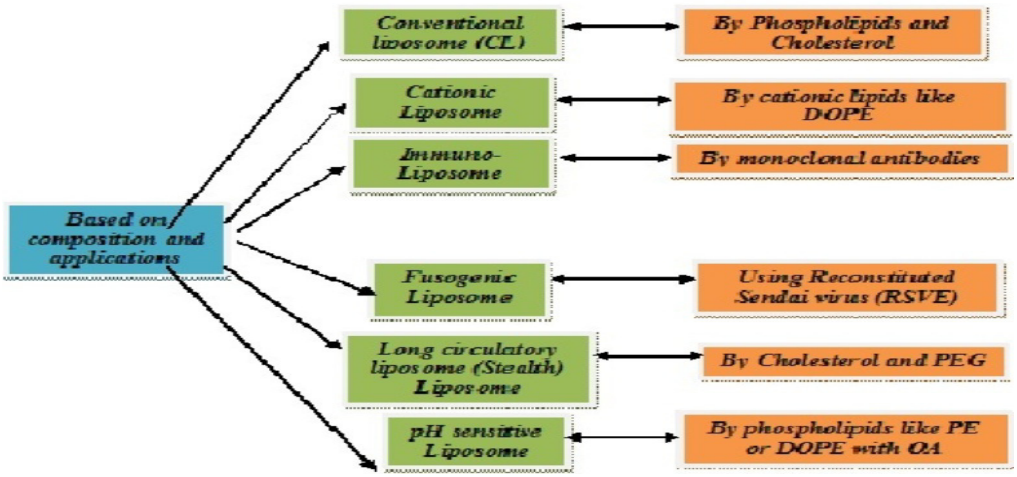

Fig.3 Classification of liposomes based on composition and applications

Liposomes as delivery systems for antibiotics

Antibiotics have always been proved of limited application in healthcare due to their toxicity or weak bio distribution and pharmacokinetics. Even though such antibiotics have potential activity, but they are only used as last chance of treatment where risk of side effects is high. Encapsulation of the drugs in lipid vesicles has opened new horizons in designing the required pharmacokinetic and pharmacodynamic properties[16, 17]

Liposome as antibiotic carriers offers following advantages:

i. Change in pharmacokinetics and biodistribution.
ii. Reduced toxic effects.
iii. Wider activity through killing of intracellular pathogens.
iv. Target specific drug delivery.
v. Increased activity by circumventing bacterial resistance.

Different types of liposomal formulations aids in designing of an effective antibiotic forms and subsequent therapeutic success[18].

Changes in pharmacokinetics, bio distribution and reduced toxic effects

The liposomal carriers offer a gradual and sustained release of antibiotics while the drug is circulating in the body, which helps in maintenance of a proper drug concentration for a relatively long term. When compared with administration of the free antibiotic, they exhibit a quick and short effect and that requires several doses per day. Drug encapsulated liposomal vesicles improves the pharmacokinetics and also helps in protection of antibiotics against the hydrolytic activity of enzymes as well as chemical and immunological deactivation[19].

Conventional liposomes injected by intravenous application are recognized as foreign matter by immunological system of body and are opsonized. These results in activation of nonspecific defence mechanisms and the liposomes are captured by the mononuclear phagocyte system (MPS), which results in lower blood circulation time and faster blood clearance. Liposomes can accumulate in the liver, spleen, lungs, and kidneys. The phenomenon of phagocytosis of liposomes is intended for destruction of intracellular pathogen[20, 21].

Wider activity through killing of intracellular pathogens

Liposomes when applied in destruction of intracellular pathogen have proved to be very successful. Liposomes were applied to various types of infections such as treatment of diseases caused by intracellular bacteria. A rigid conventional liposomal vesicle and PEG-coated ones increased the retention of drug in the proper tissues, provided sustained release, decreased toxicity, and enhanced the concentration at the site of infection. It was found that the application of liposomal forms of isoniazid, rifampin, and clarithromycin significantly enhanced antibacterial efficacy compared with the free drugs for treatment of tuberculosis[22, 23].

Liposomal formulations of antibiotics have also been extensively researched for the eradication of other obligatory and non-obligatory intracellular pathogens[24]. The first experiments involving liposomal systems for eradication of intracellular pathogen has been developed where

conventional liposomes bearing gentamicin were used for the treatment of brucellosis[16]. The increased level of liposome in spleen and liver was obtained because of uptake of liposome vesicles by MPS, which results in decrease in number of bacteria in these tissues[25, 26].

Target specific drug delivery

On-going has proved the possibility to target liposomes to particular tissues, organs, and even microorganisms[27]. Target selectivity of Liposomal drug formulations can be achieved using following ligands:

- Incorporation of specific immunoglobulins.
- By addition of proteins.
- Through specific oligosaccharide chains.
- By formulating pH-sensitive vesicles.
- By constructing thermo-sensitive vesicles.

The specific or non-specific interaction with target depends on the method for formulation of liposomes. For nonspecific action, the charge of the membrane plays an important role. For example, bacterial cells possess negatively charged surfaces, so positively charged liposomal vesicles show stronger cell interaction. In case of specific action, targeted liposome contains proteins, antibodies, or immunoglobulin fragments that binds to specifically damaged or infarcted tissues. Liposomes as drug carriers are also useful in preventing bio-film formation and treatment[28]. It has been tested for specificity and affinity of immunoliposomes to *Streptococcus oralis* biofilms[29, 30].

Increased activity by circumventing bacterial resistance

Liposomal formulations can improve pharmacokinetics of antibiotics by promoting circulation time and residence time. These vesicles can also be targeted by means of anchored ligands to particular bacteria. A lot of research describing the proper lipid formulations, drug distribution, and vesicle–bacterium interactions leading to enhancement of antimicrobial drug activity against most common extracellular bacteria, such as *P. aeruginosa, K. pneumoniae,. E. coli, Acinetobacter sp., and S. aureus* have been published[31, 32].

Fluidosomes® fluid vesicles composed of DPPC/DMPG 18:1 has been shown to be fused with the bacterial membrane of *P. aeruginosa* and releases its contents i.e. tobramycin directly into the periplasmic space which allows in achieving an antibacterial effect with a sub-MIC concentration of the drug and has increased the therapeutic index of tobramycin. For the eradication of drug-resistant *P. aeruginosa* strains, a direct interaction between liposome and bacterial cell can be promising. Bacterial resistance is developed due to lower permeability of the outer membrane or to an efficient efflux system. The application of antibiotics in liposomal vesicles could possibly overcome bacterial resistance mechanisms[33].

A DMPC/CHOL in molar ratio 2:1 containing gentamicin formulation has shown of improved killing time and enhanced antimicrobial activity[34]. Similar results in MIC reduction are also obtained for liposomes of DPPC/CHOL in molar ratio 2:1 containing amikacin, gentamicin, and tobramycin. Such experiments have also been performed with other antibiotics encapsulated in the same system of cholesterol vesicles[35, 36].

Formulations of DPPC/CHOL and POPC/CHOL containing polymyxin B has shown more potential in killing of bacterial strains than the free drug. In vitro activity has also been tested on *Bordetella bronchiseptica, P. aeruginosa, E. coli, K. pneumoniae, Acinetobacter lwoffii, and Acinetobacter baumannii* strains. Liposomal polymyxin B reduced MIC level 4–16 times when compared with the free antibiotic. The application of lipid vesicles of polymyxin B can reduce the disadvantages that may arise during the systemic use of this drug, such as nephrotoxicity, ototoxicity, and neuromuscular blockade. Liposomal form of this drug can also exhibit higher antimicrobial activity[37, 38].

Disadvantages of liposomal antibiotics

Liposomal antibiotics have short shelf-life, which limits drug stability which can be conditioned by both physical and chemical processes. Chemical instability occurs mainly due to the hydrolysis of ester bonds or oxidation of unsaturated acyl chains of the lipids. These occur in both synthetic and natural phospholipids. Stability of liposomal depends on nature of lipid composition and its storage temperature. Oxidation can be prevented by addition of antioxidant components or by freeze-drying. A low storage temperature also circumvents hydrolysis[39].

The physical instability of liposomal drugs leads to drug leakage from the lipid vesicle that is higher in the liquid than in the gel phase. The problem associated with liposomal drug stability occurs in cases of in vivo administration which can be enhanced by the addition of cholesterol that stabilises their membrane and fluidity[40, 41].

An important aspect of the physical instability of liposomes is the aggregation and fusion of liposomal vesicles that results in changes in liposome size, which affects the in-vivo therapeutic efficacy of drug. Liposomal drug formulations are only useful if there is a therapeutic amount of drug and a reasonable amount of lipids are encapsulated in proper manner. Also lipids in high doses results in toxicity and compromise the pharmacokinetics of liposomal drugs[6]. Low encapsulation also makes liposomal drug application much more expensive than conventional antibiotic treatment[42].

The preparation of liposomes at laboratory scale is expensive and complex and it is not always possible to scale-up the process[43]. Few of laboratory methods are being used in industry such as detergent removal method, ethanol injection method, and lyophilisation of bilayer-forming lipids in the presence of drug[39].

Another limitation of liposomal formulation is sterility. Sterilization procedures for liposomal antibiotics could not include the use of heat, irradiation, or chemical agents. Lipids are very sensitive to high temperatures and hence easily undergo oxidation and hydrolysis. Heat sterilization can be applied when dealing with thermo stable and lipophilic drugs[44]. A useful method of liposome sterilization is mechanical filtration, but all liposome vesicles need not be smaller than bacterial cells. Also it does not guarantee the removal of viral particles[45].

4.CONCLUSION

Antibiotics are very important class of drug useful in different types of microsomal infections. But, antibiotic therapy results in severe side effects. A liposomal drug delivery system is a novel process which can reduce the degree of drug toxicity. Target-specific delivery of some antibiotics have also been investigated which result in increased therapeutic effect and reduced dose and toxicity. Moreover, the antimicrobial efficacy of antibiotics can also increase via liposomal drug

delivery. Although there are some disadvantages associated with liposomal formulations, they can be prevented by scientific research and newer technologies. Liposomal drug delivery seems to be a promising vehicle for delivery of major antibiotics in near future.

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