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DEVELOPMENT & APPLICATION OF LIPOSOMES IN DRUG DELIVERY

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ABSTRACT:

Liposomes are a group of lipids that have been used for a variety of biological applications, includingDNA vaccination and gene therapy. Liposomal drug delivery systems have been utilized in the treatment of various diseases, including cancer, ovarian cancer, and advanced breast cancer. Several drug delivery approaches have been proposed for liposome delivery systems, including small unilamellar vesicles (SUVs),transdermal drug delivery vectors, and DNA vaccines. This review focuses on recent advances in the development and application of liposomal delivery systems. Liposomes have found widespread application in addressing both anterior and posterior segment eye disorders. In addition, they serve as immunoadjuvantsin several therapeutic contexts, such as cancer drug therapy and improved gene therapy efficiency.

KEY WORDS: Liposomes, Gene Therapy, Immunoadjuvants, Vaccine, Cancer treatment.

INTRODUCTION:

When placed in water, phospholipids spontaneously organize into enclosed structures surrounded bydouble-layered membranes, creating a system known as liposomes[1]. These tiny, spherical vesicles can begenerated from various substances such as cholesterol, safe surfactants, sphingolipids, glycolipids, fatty acids, and even membrane proteins[2]. Liposomes serve as carriers for a wide range of molecules includingsmall drugs, proteins, nucleotides, and plasmids. A.D. Bangham[3] discovered liposomes roughly 40 years ago, and since then, they've become a versatile tool in biology, biochemistry, and medicine. They've been utilized to deliver diverse compounds within their aqueous interiors since the 1960s. Liposomes can be tailored in terms of size, composition, charge, and structure. Some liposomal formulations of anticancer drugs and antifungal agents have been commercialized[4].

Liposomes first demonstrated their potential in genetic enzyme deficiency therapy during the 1970s[5,6].

Progress in enhancing liposome stability was achieved during the 1970s and 1980s, resulting in prolonged circulation times after intravenous administration and improved distribution within the body. Thevital anticancer drug doxorubicin was formulated as a liposome in the 1980s to enhance its therapeutic effectiveness. Liposomes exert various mechanisms both inside and outside the body [7]:

1. They can attach to cellular membranes and merge with them, releasing their content into the cell.
2. .Cells might uptake liposomes, integrating their phospholipids into the cell membrane, thereby releasingthe trapped drug.
3. In phagocytic cells, liposomes are internalized, and their phospholipid walls are acted upon by lysosomes,leading to the release of active pharmaceutical ingredients.
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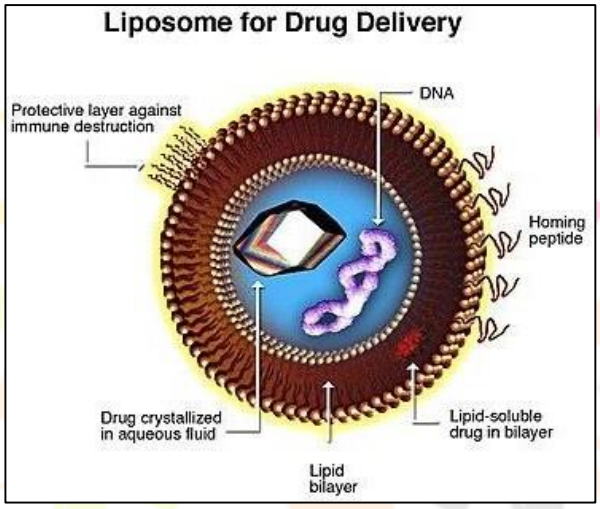

Fig.(1): liposome for drug delivery[8]

PREPARATION TECHNIQUES OF LIPOSOMES:

The traditional approach to crafting liposomes begins by dissolving lipids within an organic solvent. Following this, the solvent is allowed to evaporate, leaving behind a thin lipid film on the container's surface. Subsequently, an aqueous drug solution is introduced onto the lipid film. Agitation and sonicationare then employed to generate Small Unilamellar Vesicles (SUVs). Any drug that hasn't been encapsulatedneeds to be extracted. Occasionally, preparation methods may merge the hydration and sizing stages [9]

1.PASSIVE LOADING TECHNIQUE

The passive loading technique involves encapsulating the drug by introducing its solution either beforeor during the liposome preparation process. These methods are classified into three categories:

- Mechanical dispersion
- solvent dispersion,
- detergentremoval[10].

2. ACTIVE LOADING TECHNIQUE :

The active loading method entails loading the drug by establishing diffusion gradients between theexternal and internal aqueous phases[10].

3. METHOD OF LIPID FILM HYDRATION :

The lipid film hydration technique is widely used and represents one of the simplest ways to prepare liposomes. The procedure involves dissolving lipid components in a solvent, placing the mixture in a round-bottom flask, and subsequently evaporating the organic solvents. The flask, containing the lipid mixture, is connected to a rotary evaporator linked to a vacuum pump, and it's rotated at an appropriate speed while maintaining a temperature around 30°C.

As the process continues, a dry lipid residue accumulates on the inner walls of the flask. Rotation persistsfor an additional 15 minutes after the dry lipid residue becomes apparent. Following detachment from the evaporator, nitrogen is introduced into the flask. The residual solvent is then removed through lyophilization(freeze-drying). Nitrogen is reintroduced into the flask, and buffer is added to dissolve the lipid layer. The resulting suspension contains Multilamellar Vesicles (MLVs) liposomes.

However, this method has several limitations including low encapsulation efficiency, challenges in scaling up the process, and variations in size distribution among the liposomes[11].

4. MICROEMULSIFICATION :

This technique is employed on a large scale to create small lipid vesicles (SUVs). To begin, an inner lipid layer is formed using a w/o (water-in-oil) microemulsion. A mixture of surfactant (Phosphatidylcholine) and cosurfactant (Cremophor EL) in varying weight ratios (referred to as "comix") is combined. In separate glass vials, 10 ml of diethyl ether serves as the oil phase, and the comix is thoroughly mixed. This mixture is gradually diluted with a solution of Bovine Serum Albumin (BSA) as the water phase until it transitions from transparent to a light blue opalescence.

The next step involves creating the outer lipid layer, composed of DOPE (dioleoyl phosphoethanolamine)and DC-Chol (dimethylaminoethane)carbamoyl) cholesterol) in a weight ratio of 4:1. This outer layer is hydrated using a mixture of ethanol and deionized water (1:2, v/v) at 60ºC for 30 minutes, resulting in micelleformation. The inner and outer lipid liquids are then mixed in a round-bottomed flask, causing the micelle toflip and establish a hydrophilic group inward and a lipophilic group outward due to changes in solvent polarity.

Subsequently, diethyl ether is vacuum-evaporated at 60ºC, enhancing the micelle's membrane fluidity. Arapid second turnover occurs, during which the lipophilic group of DOPE envelops the emulsification membrane's surface within the inner micro-emulsion. This completes the formation of a lipid bilayer. The mixture is then subjected to probe sonication for 3 minutes with 30-second intervals in an ice bath, yielding smaller-sized liposomes. This method is reproducible and produces liposomes with a favorable aqueous phase [12].

5. SONICATION TECHNIQUE :

Sonication is primarily employed for the production of Small Unilamellar Vesicles (SUVs). In this process, Multilamellar Vesicles (MLVs) that have been generated using the film hydration method are subjected to sonication to adjust the liposome sizes. This can be done using either a probe sonicator or a bath-type sonicator. One notable benefit of sonication is its time efficiency. However, there are several drawbacksassociated with this technique, including limited internal volume and encapsulation efficiency, potential metallic contamination from the sonicator probe, and the coexistence of MLVs alongside SUVs [13,14].

Two distinct sonication methods are employed:

a) **Probe Sonication:**

Here, the tip of a sonicator is immersed into the lipid dispersion. The energy dissipationin this process is substantial, resulting in increased temperatures. To counteract this, the vessel must be surrounded by an ice/water bath. Extended sonication for an hour can lead to the de-esterification of over 5%of the lipids.

b) **Bath Sonication:**

In this approach, the beaker containing the liposomal dispersion is placed within a bath sonicator. Temperature regulation of the lipid dispersion is simpler compared to probe sonication. Using an inert atmosphere, the material that has undergone sonication can be safely stored in a sterile container.

6. FREEZE-THAWED LIPOSOMES:

In this technique, the process begins by rapidly freezing Small Unilamellar Vesicles (SUVs), followed bya gradual thawing. The aggregated materials are then dispersed using sonication to create Large UnilamellarVesicles (LUVs). During the freezing and thawing phases, the fusion of SUVs occurs, leading to the formationof Unilamellar Vesicles (ULVs). This fusion of SUVs can be hindered by modifying the ionic strength of thesurrounding medium or by increasing the concentration of phospholipids.

The entrapment efficiencies achieved through this method typically range from 20 to 30% [15].

7. REVERSE PHASE EVAPORATION VESICLES:

This technique involves starting with a flask containing a lipid mixture, followed by the removal of the solvent through a rotary evaporator operating under reduced pressure. In an inert atmosphere (using nitrogen),the lipids are once again dissolved in the organic phase. This phase yields the formation of reverse phase vesicles. Diethyl ether and isopropyl ether are commonly used solvents for this process.

Subsequent to re-dispersing the lipids in the aqueous phase (containing the drug to be encapsulated), a one-phase dispersion is achieved by sonication of this two-phase system under continuous nitrogen flow. Theorganic solvent is then removed using the rotary evaporator until a gel forms. The outcome of this process istermed as reverse-phase evaporation vesicles [16].

APPLICATIONS OF LIPOSOMES:

Over the past three decades, the realm of liposome investigation has significantly broadened. Today, we can manipulate liposomes to encompass a diverse array of sizes, phospholipid compositions, cholesterol compositions, and surface morphologies, making them suitable for a wide spectrum of applications [17].

Liposomes engage with cells through various mechanisms, resulting in the association of liposomalcomponents with the target cells [18].

Liposome carriers offer the potential to target the liver and spleen and differentiate between normal andtumor tissues using tomography. In the context of transdermal drug delivery systems, liposomes have significant applications. When employed for targeting tumor cells, liposomal drug delivery systems reduce toxicity and enhance drug effectiveness. Targeting liposomes to the site of action is achieved through the attachment of amino acid fragments, such as antibodies, proteins, or appropriate fragments that target specific cell receptors. Liposomal DNA delivery vectors, including advancements like LPDI-I and LPD-II,stand out as some of the safest and most versatile transfer vectors utilized to date.

Recent applications of liposomes encompass DNA vaccination and improved gene therapy efficiency.Various drug delivery approaches have been proposed for liposomal drug delivery systems, including:

i. Enhancing drug solubilization (e.g., Amphotericin-B, Minoxidil, Paclitaxel, and Cyclosporins).

ii. Protecting sensitive drug molecules (e.g., Cytosine arabinoside, DNA, RNA, Anti-sense oligonucleotides, Ribozymes).

iii. Augmenting intracellular uptake (for anticancer, antiviral, and antimicrobial drugs).

iv. Modifying pharmacokinetics and biodistribution (prolonged or sustained release of drugs with shortcirculatory half-lives).

A. LIPOSOMES FOR RESPIRATORY DRUG DELIVERY SYSTEMS

Liposomes find widespread use in various respiratory disorders. Liposomal aerosols offer severaladvantages over conventional aerosols, as outlined in reference[19] :

1. Sustained release.
2. Prevention of local irritation.
3. Reduced toxicity.
4. Improved stability within the large aqueous core.

Numerous injectable liposome-based products are now available in the market, including Ambisome,Fungisome, and Myocet. The effectiveness of a liposomal drug delivery system for the lungs depends on specific parameters:

1. Lipid composition.
2. Size.
3. Charge.
4. Drug-to-lipid ratio.
5. Method of delivery.

The recent utilization of liposomes for DNA delivery to the lungs indicates a growing understanding oftheir use in macromolecular delivery through inhalation. Much of this new knowledge, including novel lipids and analytical techniques, can be applied to the development of liposome-based protein formulations.For inhalation, liposomes can be in liquid or dry form, with drug release occurring during nebulization.
Drug-powder liposomes can be produced through milling or spray drying. The table 1 in reference listsdrugs formulated in liposomal form.

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Active constituent	**Effect**
Insuline	Facilitated pulmonary adsorption and enhanced hypoglycemic effect
Catalase	Conferred resistance to pulmonary oxygen toxicity
Super oxide dusmutase	Minimized toxicity to subsequent hyperoxia and improved survival
Cyclosporins	Preferentially adsorbd by lung and shows sustained release
Ricin vaccine	Improved safety profile for intra pulmonary vaccination

Table 1. Liposomal formulation for the respiratory disorder [20]

B. LIPOSOMES IN THE TREATMENT OF EYE DISORDERS

Liposomes have found extensive application in addressing both anterior and posterior segment eye disorders. Eye ailments encompass dry eyes, keratitis, corneal transplant rejection, uveitis, endophthalmitis, and proliferative vitreoretinopathy. Retinal diseases stand as a primary cause of blindness in developed nations. Liposomes serve as vectors for genetic transfection and monoclonal antibody-directed vehicles in these treatments.

Contemporary treatment techniques, such as the application of focal lasers to induce the release of liposomal drugs and dyes, are employed in the selective treatment of tumors and the occlusion of neovascular vessels. These techniques are instrumental in angiography, retinal and choroidal blood vessel stasis. Currently, liposomal drug formulations have received approval for two patented drugs, including 'verteporfin' for use in the eye. The potential benefits of liposomes are expected to play a crucial role in thefuture of ophthalmology in terms of treatment, diagnosis, and research[21].

C. LIPOSOMES AS VACCINE ADJUVANTS [64]

Liposomes have firmly established themselves as immunoadjuvants, enhancing both cell-mediated andnon-cell-mediated (humoral) immunity, as detailed in Table 2. Liposomes serve as immunoadjuvants in several therapeutic contexts:

1. Utilizing liposomes as immunological (vaccine) adjuvants.
2. Developing liposomal vaccines.
3. Employing liposomes as carriers for immunomodulation.
4. Using liposomes as tools in immunodiagnostics.

Liposomal immunoadjuvants function by gradually releasing encapsulated antigens upon intramuscularliposomes in lymphoid tissues is facilitated by targeting liposomes with the assistance of phosphatidylserine[22]. Liposomal vaccines can be created by introducing microbes, soluble antigens, or cytokines of deoxyribonucleic acid with liposomes, thereby stimulating an immune response through the expression of antigenic proteins. Antigens can also be covalently linked to the liposomal membrane[23].

*Ab = Antibody

Table 2. Some antigen as liposomal preparation and their applications

To regulate antigen release and enhance antibody responses, liposomes containing antigens are enclosed within alginate-lysine microcapsules for a second time. Liposomal vaccines can be safely storedunder refrigeration for approximately 12 months.

D. LIPOSOMES FOR TARGETING THE BRAIN

Antigen as liposomal preparation	Applications
Rabies glycoproteins	Interleukin-2 enhancement
Cholera toxin	Enhanced Ab*level
Diphtheria toxoid	Superior immunoadjuvant
Herpes simplex virus	Enhanced Ab level
Hepatitis B virus	Higher Ab response
Bacterial polysaccharides	Superior immunoadjuvants
Tetanus toxoids	Increased Ab titre

The biocompatible and biodegradable properties of liposomes have recently prompted research intotheir use as a drug delivery system for the brain [24].

Liposomes, whether they are small in diameter (100 nm) or large, can freely diffuse through the Blood-Brain

Barrier (BBB). However, there is a possibility that small unilamellar vesicles (SUVs) coupled with brain drug transport vectors could be transported through the BBB via receptor-mediated or absorptive- mediated transcytosis. Additionally, recently developed cationic liposomes have been shown to undergo absorptive-mediated endocytosis into cells, but it is yet to be determined if they can successfully undergo absorptive-mediated transcytosis through the BBB.

Extensive research has been conducted on the transport of substances through the BBB using liposomes. A significant finding from these studies is that the addition of sulphatide (a sulfur ester of galactocerebroside) to the liposome composition enhances their ability to cross the BBB[25]. Wang et al. reported that liposomes coated with mannose can reach brain tissue, and the mannose coating assists in transporting loaded drugs through the BBB[26]. Neuropeptides like leu-enkephalin and mefenkephalin kyo-forphin typically do not cross the BBB when administered systemically. However, the antidepressant amitriptyline can penetrate the BBB due to the versatility of this approach. Nanoparticles (NPs) were designed with various stabilizers, and it was observed that amitriptyline levels in the brain significantly increased when the substance was adsorbed onto the NPs and coated or stabilized with polysorbate 85[27].

E.LIPOSOMES IN TUMOUR THERAPY :

Long-term cancer drug therapy often results in various toxic side effects. Liposomal therapy, designed to target tumor cells, has revolutionized the field of cancer treatment with minimal side effects. Small and stable liposomes have been recognized for their passive targeting ability towards different tumors. They cancirculate in the bloodstream for extended periods and extravasate into tissues with enhanced vascular permeability[28,29]. However, the uptake of liposomes by macrophages in the liver and spleen had hindered their development as drug delivery systems for over two decades.

Preparation	Drug	Targeted Site
Liposome(Doxil)	Doxorubicin	Kaposi sarcoma
Liposome(EVACT™)	DOxorubicin	Refractory tumour Metastatic breast cancer
Liposome (DaunoXome)	Daunosome	Advanced Kaposi, sarcoma, breast, small celllung cancer, leukaemia and solid tumour
Liposome	Nystatin	Systemic fungal infection
Liposome	Anamycin	Kaposi, sarcoma, Refractory breast cancer
Liposome(VincaXome)	Vincristin	Solid tumour
Liposome (Mikasome)	Amikacin	Serious bacterial infection

Table 3. Various intravenous liposomal antibiotics / anti-neoplastics

These are long-circulating liposomes, prepared through various methods. Additionally, Caelyx and Myocet are liposomal versions of doxorubicin. Caelyx is employed in the treatment of metastatic ovarian cancer andadvanced breast cancer. Myocet is approved for use in metastatic breast cancer[30,31,32].

Conclusion: Since their discovery in the 1960s and the demonstration of their potential for drug encapsulation, liposome vesicles have garnered the interest of researchers as potential carriers for various bioactive compounds, suitable for therapeutic applications in both humans and animals. Numerous factors contribute to their effectiveness as drug delivery vehicles. Liposomes can solubilize lipophilic drugs that would otherwise be challenging to administer intravenously. The enclosed drug remains protected from metabolizing enzymes, while components like erythrocytes and tissue injection sites are shielded from direct exposure to the full drug dosage. Due to the lipophilic nature of phospholipids, liposomes can even traverse the blood-brain barrier, making it possible to formulate hydrophilic drugs that typically struggle tocross the BBB into liposomes.Furthermore, liposomes can prolong drug action by gradually releasing the drug within the body. Targeting options can alter the drug's distribution, and liposomes can also serve as adjuvants in vaccine formulations. Various methods are employed to prepare liposomes, with the film method and dehydration-rehydration method being among the most commonly used for research purposes.Stabilizing liposomes

has been a concern to ensure their optimal shelf life. Presently, stealth liposomes (Pegylated liposomes) are in development, offering extended circulation and residence time in the body.

New advancements in liposomes include their specific binding properties to target cells such as tumor cells and specific molecules. Stealth liposomes are especially useful for delivering hydrophilic (water- soluble) anticancer drugs like doxorubicin and mitoxantrone, minimizing side effects by concentrating thedrug at the action site. Another development involves bisphosphonate-liposome-mediated depletion of macrophages. Several commercial liposomal formulations have already been discovered, registered, and successfully introduced to the pharmaceutical market. The future holds even greater promise for the marketing of more sophisticated and highly stabilized liposomal formulations. In the coming years, liposomal drug delivery systems are poised to revolutionize vesicular systems, particularly in cancer treatment.

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