Liposomes As Novel Drug Delivery Agents

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Abstract **- This paper discusses the various properties of liposomes as effective new drug delivery methods. Novel drug delivery methods have revolutionized the field of chemotherapy and liposomes are at the centre of this revolution. Liposomes are small spherical vesicular structures consisting of bilayers composed of amphiphilic molecules, phospholipids and were first discussed in the 1960s. They possess similar physiochemical properties as cell membranes and thus, are able to survive in the physiological conditions of the body better than many other novel drug delivery agents. Consequently, liposomes are great tools in achieving the task of targeted drug delivery. One of the biggest advantages associated with using liposomes as drug delivery vehicles is the ease of modifying liposomes according to the demands of an effective treatment strategy.**

Index Terms **- Liposomes, cationic liposomes, immunoliposomes, pH-sensitive liposomes, long circulating liposomes, cholesterols, polyethylene glycol, sphingomyelin.**

I.INTRODUCTION

In treating a disease or condition, in addition to the type of drug used, the method used to administer the drug is also very important. The passage selected for the delivery of each drug to the target site is as important as the drug used to treat any disease. An optimal drug delivery rate is important to ensure the therapeutic effect of each drug. The conventional drug delivery methods achieve the purpose of drug delivery but cannot achieve the purpose of targeted drug delivery. Such techniques lack the ability to regulate cytotoxicity that results from the use of drugs. To overcome the problem of non-specificity and high cytotoxicity associated with the use of free drugs, new drug delivery methods have been invented which involve the use of carriers for the transportation of drugs to their target site. Many new drug delivery methods are now available, such as liposomes, silica particles, niosomes, microspheres, etc. The purpose of all of these delivery methods is to increase the therapeutic index of chemotherapeutic agents while reducing their toxic side effects.

Liposomes are spherical vesicles consisting of one or more lipid bilayers. They are desirable models of cell membrane. Liposomes were discovered in 1965. The first small molecule therapeutic to be clinically applied was lipid nanoparticles. Liposomes are used as carriers of drugs utilized in the treatment of cancer. The anticancer drugs have a narrow therapeutic index and can prove to be highly toxic to tissues and organs in the body. Use of liposomes helps resolve this issue. Kidneys, GI tract and heart are the organs mainly affected by the use of antineoplastic drugs. Since the uptake of liposomes by these organs is poor, the toxicity resulting at these sites form the use of such drugs can be minimized.

II.PROPERTIES OF LIPOSOMES

The structural component of the lipid bilayers of liposomes is phospholipid consisting of a polar head group and a non-polar tail group. The head group of these molecules is hydrophilic while the tail group made of long hydrocarbon chain is hydrophobic. In the presence of water, the polar head group lines up to face the water while the non-polar tail group lines up to face away from the water. These bilayers enclose an aqueous volume.

Liposomes as small molecule therapeutics have several unique properties that makes them the desirable vehicles for delivery of drugs. The structural composition of liposomes allows it to carry hydrophilic as well as hydrophobic molecules. They can entrap molecules of varying hydrophilicity or lipophilicity in their aqueous interior, in between the phospholipid layers or at the confluence of the two phospholipid layers. Liposomes bind to cell surfaces specifically and non-specifically. Specific binding usually involves ligand-receptor type interaction. After, liposomes reach the target site, the next step to unload the drug being carried by liposomes. Some

drugs like doxorubicin can passively cross the membrane down its concentration gradient to reach its intracellular target site. But there a number of drugs which cannot be delivered to intracellular target sites through diffusion. In case of delivery of such drugs, liposomes are taken up by the cell through endocytosis followed by the enzyme induced digestion of these vesicles in lysosomes resulting in the release of their contents in the organelle and then cytosol and hence, becoming available to all the intracellular components of a cell. This intracellular delivery of the drug depends on the nature and extent of the interaction between liposomes and target cells. This interaction is heavily influenced by the chemical composition of liposomes. The physiochemical properties of liposomes such as the size of vesicles, charge density present on the membrane surface, fluidity of the membrane, permeability of the membrane etc. determines how well liposomes will be able to interact with target cells or tissues. The permeability and fluidity of the liposome membrane have a huge effect on the function of liposomes. The fluidity of liposomal membrane determines the ease of release of the drug enclosed in these vesicles and thus has a significant impact on the therapeutic efficacy of the drug. These physiochemical properties of liposomes can be altered by changing the chemical composition of the lipid bilayers of liposomes allowing the generation of liposomes with desirable properties. Thus, liposomes can be tailored to obtain vesicles of desirable size, membrane permeability and fluidity to generate the drug carriers needed to achieve specific goals.

There are various types of liposomes available on the basis of structure and mode of action such as immunoliposomes, cationic liposomes, pH sensitive liposomes, long circulating liposomes etc.

III.IMMUNOLIPOSOMES

Immunoliposomes are a class of liposomes with antibodies conjugated to the surface of liposomal membrane capable of accomplishing the task of targeted drug delivery possible because of antibodies being antigen specific. Liposomes can be functionalized with antibodies as well as with its parts. There are a number of strategies available using which the fragmentation of antibodies can be brought about in order to obtain desired antibody fragments such as digestion by enzymes pepsin, papain or subjecting the antibodies to pH changes. Antibodies or their fragments can be attached to liposomal membranes through many ways, one of them being the conjugation of antibodies to the distal end of polyethylene glycol in the phospholipid bilayers of liposomes leaving the antigen binding sites exposed for attachment to specific antigens at target sites such as tumor cells.

IV.CATIONIC LIPOSOMES

These are a class of liposomes with cationic headgroups containing lipids used for the synthesis of liposomes. These liposomes find great use in the field of gene therapy. A number of diseases in living organisms are the results of defective genes. Cationic liposomes are considered appropriate delivery agents to be used in the transfer of required DNA sequences to target sites in order to reinstate gene expression as part of the therapy involved in the treatment of diseases resulting from genetic defects. The negatively charged nucleic acid is not enclosed in the core of the vesicle instead it interacts through electrostatic forces with cationic lipids present in the liposomal membranes.

V. pH SENSITIVE LIPOSOMES

These are the class of liposomes sensitive to pH changes which can cause these vesicles to rupture and release their contents in their surroundings. Various pH-sensitive liposomes can be obtained according to the sensitivity of liposomes to different pH values. The constituents of pH-sensitive liposomal membrane exhibit fusogenic property when the membrane undergoes changes when subjected to changes in pH in its medium. The acidic conditions resulting from changes in pH interfere with the chemistry of the phospholipid bilayers of liposomes. At acidic pH protonation of the carboxyl group of amphiphilic molecules present in the liposomal membrane occurs which leads to the destabilization of liposomes. The drug delivery mechanism of these liposomes involves their endocytosis and internalization by endosomes where its acidic pH causes these carriers to release the drugs being carried by them, intracellularly.

VI.LONG CIRCULATING LIPOSOMES

These liposomes were developed to overcome the problem of liposomes becoming the target of mononuclear phagocyte system which led to reduced half-life of liposomes in the circulatory system. Long circulating liposomes created with the objective of prolonging the life of liposomes in blood stream have their membrane surface coated with polymers such as PEG (polyethylene glycol) through covalent interaction which prevents their opsonization and thus their recognition by phagocytes. The components of PEGylated liposomes (liposomes conjugated with PEG) have minimum interaction with molecules of the circulatory system and hence can be retained in the circulatory system longer. Long circulating liposomes are preferred carriers for drugs used in the treatment of tumors. Tumor tissue have faulty vasculature design along with weak lymphatic drainage permitting the liposomes carrying anti-tumor drugs to permeate the tumor cells easily and concentrate in these tissues. This allows improved drug dosage to be received by tumor cells and thus, enhances the therapeutic effects of drugs used.

VII.PASSIVE AND ACTIVE LIPOSOMAL TARGETING

Liposomal targeting has to be kept in mind while formulating strategies and techniques for treating illnesses like tumors, cancer etc. This targeting of sites can be passive or active.

The vasculature of tumors consists of blood vessels with fenestration making them leaky. This is also associated with poor drainage of the lymphatic system in these tissues. Such faulty architecture allows liposome to pile up in these sites helping achieve the targeted delivery of appropriate chemotherapeutic agents. This type of targeting by liposomes is passive. Active liposomal targeting involves targeting receptors or antigens associated with cells or sites of interest. The chemical entities or ligands added to the membrane of liposomes interact with these receptors to achieve the goal of effective drug delivery while also ensuring enhanced chemotherapy. Immunoliposomes use the mechanism of ligand targeting in their functioning. This type of targeting has great significance in chemotherapy of cancer. Active targeting allows selective binding of these drug carriers to ligands helping accomplish the goal of enhanced therapeutic effects and reduced cytotoxicity.

Liposomes are also classified on the basis of size into small sized unilamellar liposomes, large sized unilamellar liposomes and multilamellar liposomes. The lamellar arrangement of liposomes has a significant effect on its effectiveness as a drug delivery agent. The fate of liposomes being unilamellar or multilamellar depends on the methods used for the preparation of liposomes. The unilamellar liposomes have a larger aqueous environment compared to multilamellar liposomes. Therefore, unilamellar liposomes are better suited for carrying hydrophilic molecules. Multilamellar liposomes on the other hand are suitable for the entrapment of lipophilic molecules since they are composed of two or more lipid bilayers.

VIII.LOADING OF DRUGS INTO LIPOSOMES

Drugs are loaded into liposomes after their synthesis is successively completed. So, the process of loading appropriate chemotherapeutic agents into these vesicles is independent of the place and time of their synthesis. And this is the reason why this process is called remote loading of drugs. Loading of drugs is extremely important to ensure the delivery of optimal fraction of chemotherapeutics to the target site required in the effective treatment of a disease and to prevent cytotoxicity elsewhere in the body. Loading of drugs into liposomal capsules is a process determined by the physiochemical properties of drugs as well as of liposomes. Since, liposomal membranes are composed of lipids, they are more permeable to hydrophobic substances than to hydrophilic substances. Loading of chemotherapeutics in liposomes can be passive or active. Passive loading is associated with drugs soluble in water (aqueous core of liposomes). This method is less effective compared to active loading of drugs and cannot to be used for many drugs. So, these drugs can be modified to become more soluble in water to be passively loaded into liposomes. Active loading is a more efficient way of loading drugs which ensures minimum wastage of drugs. It is driven by pH gradient existing across the liposomal membrane. The unionized drug from outside of liposomes upon being internalized becomes ionized because of subjection to changes in pH. Active loading cannot be used to transfer nonionizable drugs into liposomes. Addition of charge to the surface of such drugs allows to load them actively.

IX.RETENTION OF DRUGS IN LIPOSOMES

Retention of drugs in liposomes is dependent on the properties of drugs as well as of liposomes. Liposomes are more permeable to hydrophobic chemical species than to hydrophilic chemical species. The aqueous environment encapsulated inside these spherical capsules contributes to the ability of liposomes to retain certain drugs. The retention of drugs is also dependent on the properties of drugs to precipitate with lipids and solubilize in water. Some drugs precipitate more easily compared to others. And it is easier to retain such drugs in liposomes. The drugs which are more hydrophilic are easier to retain in the aqueous core of liposomes. The drugs loaded successfully in liposomes may leak through the fenestration present in the lipid bilayer of liposomes. In order to retain such drugs like vincristine and vinblastine, cholesterol is added to the composition of liposomal membranes making its pores tight so that substances present inside do not leak. Liposomes consisting of cholesterols in their membranes undergo destabilization under physiological conditions because of the presence of high-density lipoproteins which remove cholesterol from the liposomal membranes. This problem can be overcome by increasing the concentration of cholesterol to 30 mole % or more. Thus, increasing the concentration of cholesterols in the liposomal membrane allows them to survive the attack of high-density lipoproteins causing the removal of cholesterol from them. Use of sphingomyelins in the constitution of membranes can also help retain drugs inside liposomes efficiently. Sphingomyelin interacts with cholesterol molecules present in the membrane through hydrogen bonding which results in the reduction of membrane permeability of liposomes preventing the leakage of drugs enclosed. Enzymes can also destabilize liposomes by acting on the ester bond present between the phospholipids of the liposomal membrane.

X.RETENTION OF LIPOSOMES IN THE CIRCULATORY SYSTEM

Conventional liposomes accomplished the goal of selective drug delivery with reduced drug induced toxicity in normal healthy cells but it did not survive in the circulatory system of our body for a considerable amount of time. To deal with this problem of clearance of liposomes in blood, several methods were discovered. Such as the addition of polymers like PEG (polyethylene glycol) to the surface of liposomes which decreases the chances of liposome being subjected to phagocytosis since it does not allow opsonins to interact with liposomes. The PEGylated liposomes are commonly called stealth liposomes. They can be conjugated with antibodies or ligands to achieve greater specificity. It is not only the chemical nature of liposomal membrane that influences the retention of liposomes in the circulatory system, the size of liposomes also has an impact. After the intravenous administration of liposomes, that rate at which larger liposomal capsules are eliminated from the blood stream is higher than that of small liposomal capsules. Although, addition of sphingomyelin to the liposomal membranes helps liposomes retain drugs better but liposomes incorporated with polyethylene glycol do not support the interaction of sphingomyelin with cholesterols and this interferes with the retention of liposomes in the blood.

XI.RELEASE OF DRUGS FROM LIPOSOMES

The rate at which drugs are released from liposomes or any other drug delivery agent is important for achieving maximum efficacy of the drugs being delivered. The bioavailability of the drug at the site of target is important to ensure the desired therapeutic effects. The drugs should be delivered in sufficient amount for a sufficient period of time to its target site like tumor cells in order to be able to achieve the maximum therapeutic effects of the chemotherapeutic agent being used. Liposomes make this possible because of the ability to modify liposomes according to the requirements of desirable drug delivery methods in the treatment of diseases. The process of loading of drugs as well as the process of retaining drugs in liposomes can interfere with the of delivery of drugs. For example, doxorubicin, an anticancer drug, is loaded into liposomes with ammonium sulphate gradient which allows the drug to precipitate with the liposomal vesicles. Because of the precipitation of doxorubicin, it is released at slower rate. Although this minimizes the chances of drug caused toxicity, it also reduces the therapeutic index of the drug.

XII.CONCLUSION

The development of novel drug delivery methods has been a game changer in the field of science which helped overcome a number of difficulties associated with conventional drug delivery methods. Liposomes have been found useful in delivering many drugs as they can be tuned and modified according to the requirements. There is a plethora of studies available on liposomes with loads of information about their physiochemical properties, advantages, disadvantages, applications etc. However, there is still more room for research on liposomes and how to use them for various scientific expeditions.

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