IJPSR (2012), Vol. 3, Issue 10 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 June, 2012; received in revised form 11 September, 2012; accepted 21 September, 2012

USE OF LIPOSOMES IN CANCER THERAPY: A REVIEW

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Keywords:
Liposomal drug delivery,
Liposomes,
Liposome in cancer,
Cancer therapy

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QUICK RESPONSE CODE

IJPSR:
ICV- 4.57

Website:
www.ijpsr.com

ABSTRACT

Cancer is uncontrolled growth of abnormal cells in the body. The cells causing cancer are called malignant cells. There are so many means by which one can treat the cancer such as chemotherapy, radiation treatment, surgery, etc. With all these treatments there are some side effects as loss of hairs, living cells may get killed, for surgery skilled persons are required and chances of reoccurrences are high. As per those side effects there is less popularity of such drugs. To overcome these problems and side effects liposomal treatment is useful. Liposomal drugs have high encapsulation capacity, hence shows a significant anticancer activity with decreased toxicity preferentially cadiotoxicity. These liposomal treatments have great prolonged circulation as in daunorubicin and pegylated liposomal doxorubicin. This liposomal drug delivery also developed for delivery of various drugs. Further generation of drug delivery systems will include true molecular targeting, immunoliposomes and other ligand directed constructs that represent emphasize on biological components capable of tumor recognition with delivery technologies.

INTRODUCTION: Liposomes were first described by a British hematologist Dr. Alec D. Bangham in 1961 (published in 1964), at the Babraham Institute, Cambridge. These were discovered when Dr. Alec Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. There is a resemblance to liposome to plasmalemma and the microscope picture served as first real evidence for cell membrane being a bilayer lipid structure. The word liposome derived from two Greek words Lipo means Fat and Soma means Body, it is named so because of its composition resemblance with phospholipids. Liposome is an artificially prepared vesicle primarily composed of a lipid bilayer. Liposome is a vehicle for administration of nutrients and pharmaceutical drugs. These are composed of natured phospholipids and may contain mixed lipid chains with surfactant properties (e.g. egg phosphatidyl ethanolamine). Liposome design may contain surface ligands for attaching to unhealthy tissue. The major types of liposome are including-

1. 1] Multilamellar vesicle (MLV),
2. 2] Small unilamellar vesicle (SUV),
3. 3] Large unilamellar vesicle (LUV).

Liposomes are different than that of micelles and reverse micelles that are composed of monolayers. Drug delivery systems enhance the therapeutic index of anticancer agents. This is mainly either by increasing drug concentration in tumor cells and by decreasing exposure in normal human tissues. Hence this helps to improve the drug delivery systems especially in case of breast cancer.

Cancer [3, 4]**:** Cancer is uncontrolled growth of abnormal cells in the body. The cells causing cancer are called as malignant cells.

The common types of cancer in men and women is given in **Table 1.**

TABLE 1: THE COMMON TYPES OF CANCER IN MEN AND WOMEN

Sr. No.	Types of cancer in men	Types of cancer in women
1	Prostate cancer	Breast cancer
2	Lung cancer	Colon cancer
3	Colon cancer	Lung cancer

Some other types of cancers may include:

1. Brain cancer
2. Cervical cancer
3. Hodgkin's lymphoma
4. Kidney cancer
5. Leukemia
6. Liver cancer
7. Non-Hodgkin's lymphoma
8. Ovarian cancer
9. Skin cancer
10. Testicular cancer
11. Thyroid cancer
12. Uterine cancer etc.

Need to introduce Liposomes [5, 7, 8]**:** As here is uncontrolled cancer cell growth as they divide more rapidly than normal cells, many anticancer drugs are made to kill those growing cells. But in such case some normal, healthy cells also get multiply quickly and chemotherapy can affect those cells and shown side effect of chemotherapy. These fast growing normal cells that affected include blood cells forming bone marrow and cells in digestive tract (that are the cells at mouth, stomach, intestine, esophagus), reproductive system that are sexual organs and hair follicles. Some of the anticancer drugs may affect cells of some vital organs that are cells from heart, kidney, bladder, lungs and nervous system.

The barriers to penetration of the drug in solid tumors involve heterogeneous vascular supply and high interstitial pressures within tumor cells or tissue especially in necrotic zones. Drug delivery systems are designed so as to get slow release of macromolecular agents through tumor cells or tissue. These systems are made to enhance permeability and retention effect at tumor tissue. These are due to dysregulated nature of tumor angiogenesis that characteristically involves structural and physiological defects leading to hyperpermiability. High molecular size molecules having highly restricted volumes of distribution and capacity for greatly prolonged circulation will preferentially extravasate from those abnormal vessels and accumulate in tumor cells or tissues.

Anatomy of Liposome [6, 7, 8]**:**

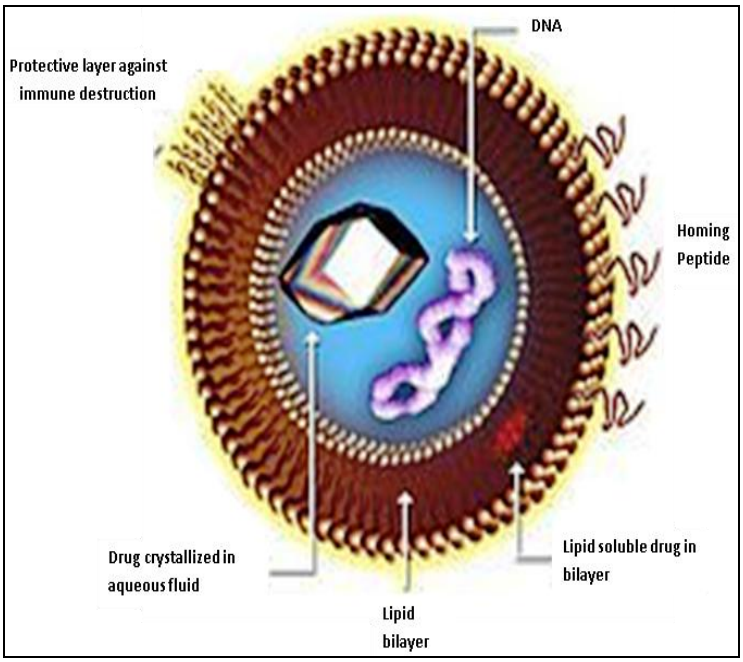

FIG. 1: STRUCTURE OF LIPOSOME

A liposome is a tiny bubble or vesicle, made of cell membrane. These liposomes can be filled with drugs and hence are the choice for treatment for some diseases and cancer. The membrane of liposomes made of phospholipids that are having head group and tail group. The head groups are hydrophilic and the tail groups are hydrophobic due to a log hydrocarbon chain. In nature, phospholipids found in stable membrane composed of two layers. In presence of water, as hydrophilic in nature of head groups they are getting attracted to the water and line up to form a surface like structure away from water.

In a cell as there is a bilayer one head group is attracted towards the outside water while another get attracted towards the water present inside the cell. The hydrophobic tail groups face one another hence forming a bilayer. When the membrane phospholipids get disturbed, they themselves get resembled into tiny spheres; their size is smaller than that of a normal cell either as bilayers or monolayers. The bilayers formed are called as liposomes and the monolayers are called as micelles. The lipids present in plasma membrane are mainly phospholipids as phosphatidylethanolamine and phosphatidylcholine. These phospholipids are amphiphilic and the hydrocarbon tail of the molecule is hydrophobic and its polar head hydrophilic. In the composition of liposomes phospholipids are mixed lipid chain and pure surfactant components like DOPE (dioleoylphosphatidylethanolamine).

Liposomes and Cancer [10, 11, 12]**:** Liposomes are having property or natural ability to target cancer. The endothelial walls of all healthy human blood vessels are encapsulated by endothelial cells bounded together by tight junctions. These tight junctions help to stop the large particle in blood from leaking out of the vessel. Such type of arrangement is not there in case of tumor vessel and hence is diagnostically "leaky". This ability is known as enhanced permeability and retention effect. Liposomes of size less than 400 nm, can rapidly enter tumor sites from blood, but these are then kept in bloodstream by endothelial wall in healthy tissue.

Design of Liposome [6, 7, 13, 16]**:** Liposome contains aqueous solution region that is encapsulated inside a hydrophobic membrane, hence dissolved water soluble solute can not easily pass through the lipids. Hence hydrophobic drugs can be dissolved into the membrane, so in this way liposome can carry both hydrophobic as well as hydrophilic molecules. For the drug delivery at the site of action, this lipid bilayer fuses with other bilayer of cell membrane and deliver the contents from liposome. To deliver the drug past the lipid bilayer one can make liposomes in a solution of DNA that are unable to diffuse through the membrane. Liposome does not have lipophobic contents such as water, although it usually does. Hence liposomes are used as models for artificial cells.

Liposomes containing low or high pH can be constructed such that the aqueous drug which is dissolved will be charged in solution. The pH within the liposome naturally neutralizes, hence the drug inside it also neutralized, allowing it to pass through the membrane easily. Hence liposomes work by diffusion rather than by direct cell fusion to deliver the drug. Similar approach can be developed by detoxification of drug by injecting empty liposomes with a transmembrane pH gradient. In such conditions vesicles act as sink to scavenge the drug in the blood circulation and prevent its toxic effect. Another approach for liposome drug delivery is to target endocytosis events.

Liposomes can be made in particular size range making them viable targets for natural macrophage phogocytosis. As a result the liposomes are getting digested while in the macrophage's phagosome, releasing its drug. Liposome can also be combined with opsonins and ligands to activate endocytosis in other cell types. The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection. In addition, liposomes can be used as carriers for the delivery of

- Dyes to textiles,
- Pesticides to plants,
- Enzymes and nutritional supplement to foods,
- Cosmetics to the skin.

Liposomes are also used as outer shells of some microbubbles contrast agents used in contrast-enhanced ultrasound.

Liposome Preparation [6, 7, 18, 19, 20]**:** Parameters for the liposome preparation method-

1. The physicochemical properties of the material to be entrapped and those of the liposomal ingredients.
2. Nature of the medium in which lipid vesicles are to be dispersed.
3. The active concentration of the entrapped substance and its potential toxicity.

4. Processes involved during delivery of the vesicles.

5. Optimum size, polydispersity and shelf life of vesicles for intended application.

6. Batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

The formation of liposomes and nanoliposomes are not a spontaneous process. For the formation of lipid vesicles phospholipids such as lecithin are placed in water and hence form one bilayer or a series of bilayers, each separated by water molecule, once enough energy is supplied. Another method to prepare liposomes is by sonicating phospholipids in water and this technique is used to produce materials for human use.

Mechanism [21, 22, 23]**:** The method to load drugs into liposomes depends upon the properties of drug and the lipids. Hydrophobic drugs can partition into the lipid hydrocarbon region while hydrophilic drugs trapped in the interior aqueous compartment. In reality, there are very few drugs which can segregate into hydrocarbon or aqueous compartments, e.g. AmpB- associates with lipid membrane. Hence, partitioning of AmpB into its exchange rate out of the liposome membrane is highly dependent on lipid composition. If negatively charged lipid is incorporated, it increases the stability of association with the membrane. High drug-to-lipid ratios and trapping efficiencies independent of lipid composition can be achieved with pH gradient loading techniques. Weak bases can be accumulated in liposomes according to transmembrane pH gradient. Some examples of drugs with their API available in the market are given in Table 2.

TABLE 2 : SOME EXAMPLES OF DRUGS WITH THEIR API AVAILABLE IN THE MARKET

Sr. No.	Type of drug	Name of API
1	Anthracyclines	Doxorubicin, Daunorubicin
2	Vinca alkaloids	Vincristine
3	Camptothecins	Topotecan

For doxorubicin, drug-to-lipid ratio 0.2 mol/mol, corresponding to about 20,000 molecules per 100 nm LUV can be obtained. As the drug is actively taken up into liposomes, the pH loading technique referred to as active or remote loading techniques.

In the preparation of liposome's two steps are involved i.e.-

1. Generation of pH gradient with low intraliposomal pH

2. Subsequent loading of the drug.

Transmembrane proton can be gradient generated by various ways. Liposomes can be prepared in low pH buffer e.g. pH 4 citrate buffer that is followed by exchange of external buffer solution against pH 7.5 buffer. In other way ionophores used in combination with cation gradients (high internal cation gradient). In such case the ionophores used are nigericin and A23187 which couple the outword movement of monovalent or divalent cations respectively, to inword movement of protons hence acidifies the liposome interior.

Finally, the preparation of liposomes carried out in the presence of high concentrations of weak base e.g. ammonium sulfate. Removal of external ammonium salt solution gives rise to generation of pH gradient according to same principle that is also responsible for drug loading process. For the loading of doxorubicin there is formation of drug-metal ion complex in liposome interiorcan being done. In such case the complex formation will become the driving force for accumulation.

Liposome Clearance from the body [12, 13, 14, 19]**:** After the intravenous injection of liposome in the human body, liposomes become coated by proteins circulating in the blood. Some of these proteins compromise the integrity of lipid bilayer hence causes leakage of liposome contents, while others promots recognition and elimination of liposome from blood e.g., Liposome consisting of unsaturated lipids such as EPC rapidly looses membrane integrity through lipid transfer to lipoproteins and disintegrates. These processes include insertion of ApoA1, apolipoprotein found predominantly in high density lipoprotein fraction, into lipid bilayer. Otherproteins such as opsonins mark liposomes for removal through phagocytic cells e.g. Opsonins include components of complement system (C3b, iC3b), IgG, β2 glycoprotein 1 and fibrinectin.

The removal of liposomes is carried out by mononuclear phagocyte system (MPS), in particular Kupffer cells, spleen, lung and bone marrow. The bulk of liposome injected accumulates in the liver and spleen.

Drugs available in Liposome Drug Delivery [24, 25, 26]: List of clinically approved liposomal drugs is given in **Table 3**.

TABLE 3. LIST OF CLINICALLY APPROVED LIPOSOMAL DRUGS

Sr. No.	Name	Trade name	Route of Administration	Company	Dosage Form	Indication
1	Liposomal amphotericin B	Abelcet	Parenteral	Enzon	Injectable liquid	Fungal infections
2	Liposomal amphotericin B	Ambisome	Parenteral	Gilead Sciences	Injectable liquid	Fungal and protozoal infections
3	Liposomal cytarabine	Depocyt	Parenteral	Pacira (formerly SkyePharma)	Injectable liquid	Malignant lymphomatous meningitis
4	Liposomal daunorubicin	DaunoXome	Parenteral	Gilead Sciences	Injectable liquid	HIV-related Kaposi's sarcoma
5	Liposomal doxorubicin	Myocet	Parenteral	Zeneus	Injectable liquid	Combination therapy with cyclophosphamide in metastatic breast cancer
6	Liposomal IRIV vaccine	Epaxal	Parenteral	Berna Biotech	Injectable liquid	Hepatitis A
7	Liposomal IRIV vaccine	Inflexal V	Parenteral	Berna Biotech	Injectable liquid	Influenza
8	Liposomal morphine	DepoDur	Parenteral	SkyePharma, Endo	Injectable liquid	Postsurgical analgesia
9	Liposomal verteporfin	Visudyne	Parenteral	QLT, Novartis	Injectable liquid	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
10	Liposome-PEG doxorubicin	Doxil/Caelyx	Parenteral	Ortho Biotech, Schering-Plough	Injectable liquid	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer
11	Micellular estradiol	Estrasorb	Parenteral	Novavax	Injectable liquid	Menopausal therapy

Advances in Liposome Therapy [24]: In case of advances in liposomal research is to avoid its detection by the body's immune system, especially, the cells of reticuloendothelial system (RES). Such liposomes called as "stealth liposome' and they are constructed with PEG studding the outside of membrane.

PEG coating allows longer circulatory life for drug delivery mechanism. Actually, research are going on to find out how actually PEG coating and PEG hinder or retard the binding of liposomes have some sort of biological species attached as a ligand to liposome to enable binding via expression on the targeted drug delivery site. Those targeting ligand may be monoclonal antibodies, vitamins or specific antigens.

These targeted liposomes can target nearly any type of cell present in the body and deliver the drug at the site of action. Naturally toxic drugs act as less toxic when they are delivered to diseased tissues. Similar to this way polymerosomes can also be used.

CONCLUSION: In the cancer therapy liposomal and immunoliposomal formulations plays vital role. It gives the progress in the fields of liposome technology and antibody engineering combinely with in deep analyses of drug delivery, release and its uptake *in vitro* and *in vivo* resulted in better understanding of mechanisms and requirements for targeted delivery.

The combination of doxorubicin-loaded liposomes based on PEGylated liposomes and small recombinant antibody fragments already one of the choices of route to clinical development. With new targets and evaluation the application of liposomes have been expanded to novel therapeutic concepts in cancer therapy, e.g. vascular targeting and gene silencing approaches. Further studies on this demonstrate efficacy and safety. It clears that in future, liposomes will complement the therapeutic arsenal for fighting cancer.

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How to cite this article:
Pawar HR, Bhosale SS, Derle ND: Use of Liposomes in Cancer Therapy: A Review. *Int J Pharm Sci Res*. 3(10); 3585-3590.