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Liposome Based Drug Delivery Systems in Cancer Treatment

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ABSTRACT:-
Nanoparticles as a drug delivery system have received much attention in recent years especially for cancer treatment. Liposomes are the most common and well-investigated nanocarriers for targeted drug delivery. Cancer is a life threatening disease, it is a leading cause of death worldwide, according to nearly 10 million deaths in 2020. Cancer arises from the transformation of normal cells into tumor cells in a multistage process that generally progresses from a precancerous lesion to a malignant tumor. The first line treatment of cancer is the surgical removal of solid tumor,radiation therapy & chemotherapy. These treatment strategies fail to control metastatic tumors that have reached distant organs. The success rate of chemotherapy remains low because of limited availability of drugs to the tumor tissue, their intolerable toxicity and also normal cells too damaged. The aim of newer approaches in cancer treatment is to prevent the damage of normal cells and overcome drug resistance. For cancer treatment liposomes are considered to be the most successful drug carrier system towards the targeted cancerous cells (prevent normal cells damage) known to date. Liposomes are self-assembled, uni-lamellar or multi-lamellar spherical vesicles primarily composed of phospholipids from either plant or animal origin. Liposomes encapsulate hydrophilic drugs within the aqueous core and hydrophobic drugs in the lipid bilayer, which protects the drugs from the environmental degradation during systemic circulation. They have improved therapies for a range of biomedicalapplications by stabilizing therapeutic compounds, overcoming obstacles to cellular and tissue uptake, and improving biodistribution of compounds to target sites in vivo.
Keywords :- Cancer, Chemotherapy, Toxicity, Liposomes, Tumor targeting, Pharmacokinetic, Bioavailability.

I. INTRODUCTION : -

What is cancer ? and how does it arise ?

Cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs, the latter process isreferred to as metastasis.

Risk factors of cancer :-

Diet and physical inactivity	1. Overweight and obesity 2. Low fruit and vegetables intake 3. Physical inactivity cause prostatecancer
Addictive substance	1. Smoking 2. Alcohol
Sexual and reproductive health	Unsafe sex - sex with an infected partner without any measure to prevent infectioncause cervix uteri cancer.
Environmental Risk	Urban air pollution causes lung cancer- Estimated yearly average particulate matter concentration for particles with aerodynamic diameter < 2.5 microns or 10 microns. Indoor smoke from household use of solid fuels causes lung cancer.
Other selected risk	Contaminated injections in health care settings- contamination refers to potentialtransmission of hepatitis c virus which causes liver cancer.

Tumor development :- 12

The term "cancer " refers to more than 100 forms of the disease. Almost every tissue in the body can spawn malignancy. Epithelial cancers are the most common malignancies and are called carcinomas. The creation of a malignant tumor in

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epithelial tissue.
Tumor Development begins when some cell with a normal population sustains a genetic mutation that increases its propensity to proliferate when it would normally rest.
The altered cell and it's descendants continue to look normal but they reproduce too much ,a condition termed hyperplasia. After years, one in a million of these cells suffers another mutation that further loosen control on cell growth.
In addition to proliferating excessively, the offspring of the cell appear abnormal in shape and in orientation, the tissue is now said to exhibit dysplasia. Once again after a time, a rare mutation that alters cell behavior.

The affected cells become even more abnormal in growth and appearance. If the tumor has not yet broken through any boundaries between tissues, it is called in situ cancer. The tumor may remain contained identificall, however some cells may eventually acquire additional mutations.

If the genetic changes allow the tumor to begin invading underlying tissue and to shed cells into the blood or lymph the mass is considered to have become malignant.

The renegade cells are likely to establish new tumors that are metastasized through the body ; these may become lethal by disrupting a vital organ.

Beige - Normal cells
Orange - Sustain genetic mutation
Pink - Suffered another mutation
Purple - Alter cell behavior
Blue - Acquire additional mutation

Treatment strategies :- 2

Cancer screening and early detection	George Papanicolaou identified pap test potentials for early detection of cervical cancer. In the 1960s mammography was developed for identification of breast cancer. Later early detection of cervix, breast,colon, rectum,endometrium,prostate, thyroid, oral cavity, skin, lymph nodes, ovarian etc cancers were identified and practiced in the clinic.
Surgery	After anesthesia was invented in 1846 ,surgeons Bilroth ,Handly and Halsted led cancer operations by removing the entire tumor together with lymph nodes. Later Paget Surgeon reported that cancer cells were spread from the primary tumor to other places through the bloodstream.
Hormonal Therapy	New classes of drug (aromatase inhibitors, LHRH analogues) are being used to treat prostate and breast cancer. How hormones influence growth of cancer has guided to reduce the risk of breast and prostate cancer.
Radiation Therapy	a) Conformal proton beam therapy - Proton beam used for killing tumor cells instead of x-rays. b) Stereotactic surgery and stereotactic therapy - Gamma knife can be used to deliver and treat common brain tumors. c) Intraoperative radiation therapy - cancer has been removed surgically followed by radiation to theadjacent tissue.
Immunotherapy	Use of biological agents that mimic some of the natural signals that the body uses to control tumor growth is called Immunotherapy. These natural biological agents can now be produced in the laboratory including interferons, interleukins, cytokines, antigens etc. At present scientists are developing vaccines to boost the body's immune response.
Chemotherapy	During the last decade of the 20th century, Surgeons developed new methods for cancer treatment by combining surgery with chemotherapy and radiation. Overthe years, use of many chemotherapy drugs has resulted inthe successful treatment of many types of cancers.

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Liposome:- 9 , 11 , 10,

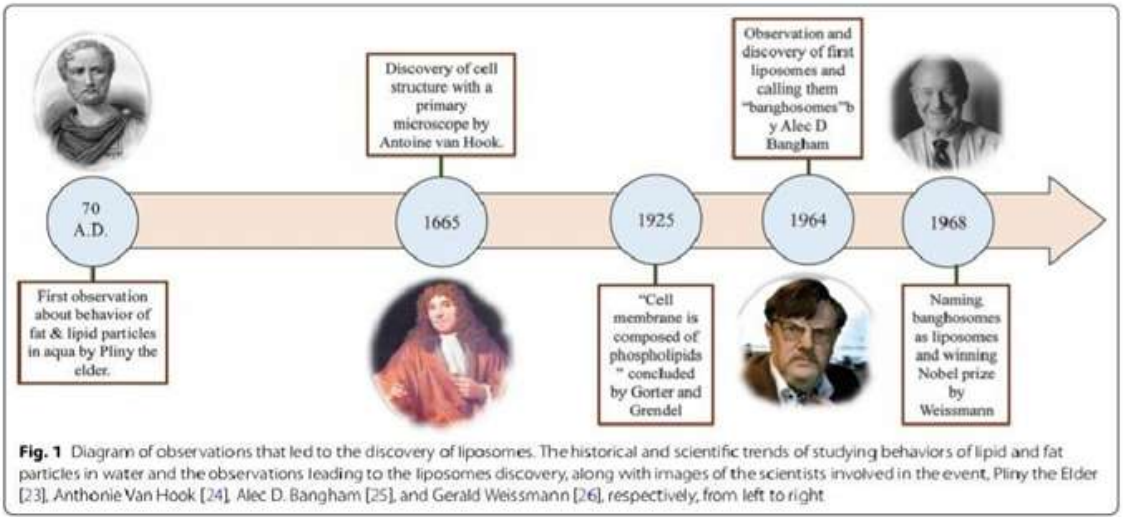

Fig. 1 Diagram of observations that led to the discovery of liposomes. The historical and scientific trends of studying behaviors of lipid and fat particles in water and the observations leading to the liposomes discovery, along with images of the scientists involved in the event, Pliny the Elder [23], Anthonie Van Hook [24], Alec D. Bangham [25], and Gerald Weissmann [26], respectively, from left to right

Liposomes are spherical, self-closed structures formed by one or several concentric lipid bilayers with an aqueous phase inside and between the lipid bilayers. There are a number of different types of liposomal vesicle:

- Multilamellar vesicles: these range in size from 500 to 5,000 nm and consist of several concentric bilayers.
- Small unilamellar vesicles: around 100 nm in size and formed by a single bilayer.
- Large unilamellar vesicles: range in size from 200 to 800 nm.

*Long-circulating liposomes : liposomes modified in such a way (usually surface-grafted with certain polymers) that they can stay in the blood much longer (for hours) than non-modified liposomes.

*Immunoliposomes: liposomes carrying antibodies attached to their surfaces and able to accumulate in the area within the body where an attached antibody recognizes and binds its antigen.

Liposomes generally reach their site of action by extravasation into the interstitial space from the bloodstream. Liposomes can target specific tissues through both active and passive targeting strategies (Figure 2). This is because liposomes can easily be manipulated by adding additional molecules to the outer surface of the lipid bilayer. Because liposomes are of the order of 400 nm in size, they are rapidly cleared by the MPS system. Reducing opsonization of liposomes by PEGylation therefore reduces clearance by the MPS, increasing the circulation half-life.

Opsonization presents such a problem to the development of therapeutically useful liposomes that nearly all research reported in the literature involves PEG-coated or PEGylated liposomes.

Liposomes are firmly established with the success of Doxil1 and liposomal formulations of other anticancer drugs are now being intensively explored to improve chemotherapy outcomes and reduce toxicity.

Attractive biological properties of liposomes:

- Liposomes are biocompatible.
- Liposomes can entrap water-soluble (hydrophilic) pharmaceutical agents in their internal water compartment and water-insoluble(hydrophobic)pharmaceuticals into the membrane.
- Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions, yet do not cause undesirable side reactions.
- Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments.
- Size, charge and surface properties of liposomes can be easily changed simply by adding new ingredients to the lipid mixture before liposome preparation and/or by variation of preparation methods.

Need of study :- 6,2

Limitations of conventional chemotherapy:-

The treatment of localized and metastasized cancers with the use of chemical

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antineoplastic drugs. These drugs are mostly administered through IV regimens which are referred to as chemotherapy. It is the primary therapeutic approach.

1. **The lack of specificity towards the neoplastic tissue.**

It cause significant damage to non-cancerous cells leading to side effects such as,

- Suppression of bone marrow activity
- Mucositis
- Nausea
- Secondary neoplasm
- Infertility

The high distribution volume of chemotherapeutic makes the drug delivery non specific to tumors. It results in an abnormal concentration of the antineoplastic drugs in healthy tissue.

2. **The lack of selectivity in mechanisms of action.**

It is a prominent drawback in conventional chemotherapy. Cytotoxic and cytostatic mechanisms induced by chemotherapeutic drugs hit healthy non cancerous tissue as well.

eg. Epirubicin (EPI) - Used in treatment of hepatocellular carcinoma (HCC) - DNA damage -Apoptosis occurs

Long term clinical use of EPI is limited due to serious non-specific toxicity to normal tissue particularly cardiac toxicity.

3. **Cytotoxicity due to high pharmacokinetic volume of the distribution for low molecular- weight drugs.**

Chemicals of low molecular weight are excreted quickly due to the fact that a higher concentration is required to achieve a therapeutic effect that leads to toxicity. The low therapeutic index of chemotherapeutic drugs implies that the needed concentration for the effective treatment is often high leading to systemic dose dependent side effects.

4. **Formulating chemotherapeutic drugs is challenging due to their poor aqueous solubility.**

The low solubility makes preparation of drugs difficult. Due to high hydrophobicity and poor solubility in water (<0.5mg/L) the chemotherapeutic application of paclitaxel has been limited. The poorly soluble drugs may cause embolization of blood vessels upon IV injection due to aggregation of the insoluble drugs that often causes local toxicity as a result of high drug concentration at the site of deposition.

eg. The currently available formulation paclitaxel comprise of - Chromophore E1+Dehydrated ethanol

Chromophore E1 (polyethoxylated castor oil) is known to be toxic and causes serious side effects including hypersensitivity reaction, nephrotoxicity and cardiotoxicity.

Surfactants may be employed in the formulations to solubilize the drug but this may cause the drugs to precipitate in vivo ,their critical micelle concentration in physiological fluid are too low to hold micellar structure capable of maintaining the drugs in solubilized state.

5. **Chemotherapy experiences limited efficacy of anticancer drugs due to strong innate or acquired chemoresistance mechanisms**.

The interstitium of a tumor tissue is characterized by high hydrostatic pressure - leading to an outward convective interstitial flow - that can remove the drug away from the tumor unlike normal tissues.

Even if the drug is successfully delivered to the tumor interstitium, it's efficacy may be limited if the cancer cell has acquired multidrug resistance. The characteristic feature of MDR is over - expression of the plasma membrane P-glycoprotein, which is capable of keeping drugs away from the cells. Several strategies have been proposed to avoid p-gp mediated MDR including the encapsulation of anticancer drugs in nanoparticles and the co-administration of p-gp inhibitors.

6. **Conventional chemotherapy encounters challenges during transport of the drugs to the tumors.**

Physicochemical properties of the drugs, including size, surface composition, charge. Charge plays a major role in transport. The physiological tumor heterogeneity inhibits a uniform drug delivery into the whole tumoral mass.In addition acidic tumor microenvironment causes degradation of the acid sensitive drugs.

Targeting of the tumor using liposome :- 4,6,8,3

Most of the APIs used in chemotherapy have been highly cytotoxic to cancer and normal cells. Therefore, they suffer from plenty of side effects and limitations, as the free drug is administered directly into the bloodstream that circulates the body. The chemotherapeutic agent

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can then be uptaken by cancer and normal tissue, leading to severe toxicity to different body organs such as heart, kidney, liver, and others. As a result, sometimes the highest possible dose of chemotherapy is administered to the patient to maximize the quantity of the medication taken up by the carrier cell. The encapsulation of chemotherapeutic agents within liposomal structure can limit the normal tissue uptake of the medication and thus improve its therapeutic index.

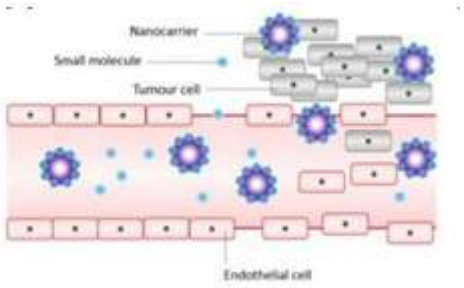
Drug delivery strategies :-

passive	Active	physical
EPR effect localized delivery	vascular endothelium Tumor cells	ultrasound Magnetic field

1. Active and passive targeting

Passive targeting, liposomes can concentrate preferentially on the tumor via. the enhancedpermeability and retention (EFR) effect of the vasculature in which leaky tumor vessels unite with absent lymph drainage. In other words, Passive targeting of liposomes happens by transferring them into the tumor interstitium via. leaky tumor vasculature through molecular movement within fluids. Liposomes can actively target tumor tissues using the antibody-based approach. This can be done by adding certain antibodies to the liposomal surface called immunoliposomes (ILP) which are specific to the cancer cells or to the endothelial cells of the tumor vasculature.

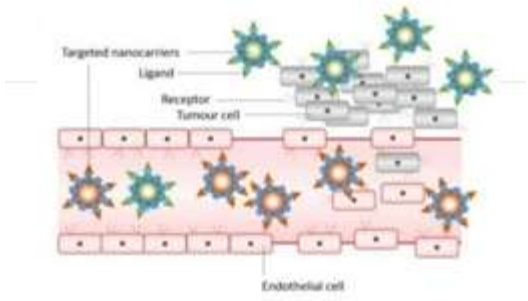
A) Passive targeting



Owing to the leaky nature of the tumor-associated blood vessels, biomacromolecules and nanosized drug delivery systems readily translocate across the capillary endothelium and enter the interstitial space. The size of the gaps between the endothelial cells lining the tumor capillaries ranges from 100 to 780 nm depending on the cancer type, as opposed to that in a typical normal endothelium of 5–10 nm [7]. In addition, solid tumors lack adequate lymphatic drainage. Therefore, there is limited circulatory recovery of the extravasated molecules, resulting in the accumulation of macromolecules and nanoparticles in the tumor microenvironment. This well-established phenomenon that leads to a nanopreparation's accumulation in the tumor microenvironment has been termed the enhanced (Figure 2) [8–12]. Utilization of the EPR effectis an effective strategy for targeting nano preparations, such as liposomes, to the site of a tumor. Unlike liposomes and other nanoparticles, low-molecular-weight drugs are not retained in the tumor site for a longer period of time since they re-enter the circulation primarily via diffusion. Targeting of these drugs relies solely on the pathophysiological properties of the tumor tissues, and is referred to as `passive drug targeting.

The accumulation of liposomes in the tumor strongly depends on the size of the endothelial gaps in the capillary vasculature for a particular cancer.

B) Active targeting



In general, actively targeted liposomes are designed to minimize off-target effects. Actively targeted liposomal systems are prepared by conjugating targeting moieties, including small-molecule ligands, peptides and monoclonal antibodies, on the liposomal surface. For example, certain receptors, such as folate and transferrin (Tf) receptors (TfR), are overexpressed on many cancer cells and have been used to make liposomes tumor

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cell specific. Liposomes that accumulate in the tumor microenvironment can be subsequently endocytosed into the cells by interacting with specific cell surface receptors . To efficiently target liposomes to cancer cells, it is necessary to link the targeting moiety in sufficient quantities to have optimum affinity for the cell surface receptors.

Nanocarriers reach tumours selectively through the leaky vasculature, or in other cases, where the nanocarrier size determines the retention in the tumour tissue. Drugs in the absence of nanocarriers diffuse freely in and out the tumour blood vessels due to their small size, and therefore their effective concentrations in the tumour decrease rapidly. The EPR effect is where drug-loaded nanocarriers cannot diffuse back into the blood stream due to their large size, resulting in progressive accumulation. In active targeting, ligands grafted at the surface ofnanocarriers bind to receptors (over)expressed by cancer cells or to angiogenic endothelial cells. Adapted and reproduced with permission.

In 1919 developed the pendant type ILP (34A-PEG-ILP)
Long circulating polyethylene glycol (PEG) ILP - attached to the antibodies (34 A antibody) at the distal end of PEG chain - These ILPs showed high targetability to the site of action more than ordinary liposomes.

These is mainly caused by the effect of free PEG ,which successfully helped to avoid
the RES uptake of the ILPs.The limitation of this approach is that not all tumor tissues or cells have a specific antigen for the targeted antibody to bind to, accordingly this approach is limited to the antigen antibody specification.

2. **An additional targeting method has been developed that uses an external trigger to solve this problem**.

This can be done by triggering the release of the chemotherapeutic agent within the interstitium after accumulating on the tumor tissue. This can be achieved by releasing the agent within the tumor vasculature using liposomes particularly designed to respond to a precise external trigger (eg.heat). For instance thermosensitive liposomes that can be administered systemically were developed. There are several strategies by which local hyperthermia could improve the effectiveness of the liposomal formulations for drug delivery.

- By inducing drug release at a temperature close to that of the lipid phase transition of the liposomes.
- By promoting blood supply to the site of action.
- By improving liposomes accumulation at the site of action by increasing endothelial permeability to liposomes.
- By increasing the permeability of the target cells to API that release from the liposomes.
- By improving drug release from liposomes by reducing the local ph of the target site of action.

3. **It was suggested by Magin et al. (1986) that the clearance and distribution of temperature-sensitive liposomes is size-dependent.** The size range of 50–200 nm was recommended , as the endothelium tissues in the kidney glomerulus have a pore size of 40–60 nm. However, macrophages in liver and spleen can easily remove these liposomes from blood circulation, as the pore size of sinusoidal endothelium in liver and spleen is around 150 nm. More studies demonstrated that vesicle size, lipid composition, surface coating and charge, and liposome-plasma protein interaction all have an impact on the clearance pharmacokinetics of liposomes by the reticuloendothelial system. Therefore, the selection of the most appropriate lipids (e.g., lysolipid temperature-sensitive liposomes), incorporation of cholesterol (to increase vesicles stability and reduce drug leakage) , and the use of optimum polymers for coating can help improve this DDS. For instance, coating PEG onto liposomes is a helpful approach that can prevent liposome engulfment by the macrophages and thus increase their blood circulation time.

4. **Another approach for the delivery of anticancer drugs is using enzyme-responsive liposomes.**The idea for this approach came after detecting high concentrations of certain enzymes in patients diagnosed with cancer. For instance, some extracellular enzymes, e.g., secreted phospholipase A2 (sPLA2) (raises in prostate [69], breast and pancreatic cancers), matrix metalloproteinases (MMPs) (specifically, MMP-2 and MMP-9 elevates in breast, colorectal, pancreatic, and lung tumours), urokinase plasminogen activator (uPA) (elevated in a number of human cancers, such as breast, colon, bladder, and

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ovarian tumours), elastase (found in high concentration in cases of lung, breast , and skin tumours), prostate-specific antigen (PSA) (raises in case of prostate tumour), and some intracellular enzymes, e.g., cathepsin B (elevated in brain, breast, prostate, and lung cancer.

5. **PEGylated, sterically stabilised liposomes can stabilise the entrapped API, enhance activity, change API disposition, and reduce toxicity.** However, they are unable to control their drug release kinetics. Overexpression of the over-mentioned enzymes could be an effective target for controlling drug release from liposomes. This was reported for DOX-loaded sPLA2- responsive liposomes developed by Mock et al. (2014) for the treatment of prostate cancer. Liposomes were prepared with cholesterol, DSPC, DSPE,and (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol) 2000)] (DSPE-PEG2000) (10 µmol total phospholipid). Animal tests showed that the liposomes were 1.5 to 2 times more effective than sterically stabilised liposomes (composed of DSPC, cholesterol, and DSPE-PEG2000) at reducing tumour growth. The mechanisms mediating enzyme and drug uptake, toxicity, and disposition of liposomes are cell- and formulation-dependent.

Different types of liposomes used in cancer cells targeting :- 5

1. Stimuli responsive liposomes
2. PH sensitive liposomes
3. Thermosensitive liposomes
4. PEGylated stealth liposomes
5. Immunoliposomes
6. Cationic liposomes
7. Fusogenic liposomes

The efficient systemic therapy of cancer is almost impossible due to harmful side effects of anticancer agents to the healthy organs and tissues. Furthermore, several problems such as low bioavailability of the drugs, low drug concentrations at the site of action, lack of drug specificity and drug-resistance also cause many restrictions on clinical applications of these drugs in the tumor therapy. Different types of the liposomal formulations have been used in medicine due to their distinctive advantages associated with their structural flexibility in the encapsulation of various agents with different physicochemical properties.

1. Stimuli responsive liposomes

Strategies used to enhance liposome-mediated drug delivery in vivo include the enhancement ofstability and circulation time in the bloodstream, targeting the specific tissues or cells, and facilitation of intra-cytoplasmic delivery. First-generation liposomes have emerged as one of the first nano-medicines used clinically for localized delivery of chemotherapy. Second-generation liposomes, such as stimuli-responsive liposomes, have the potential not only to provide site-specific chemotherapy, but also to provide triggered drug release and thus greater spatial and temporal control of therapy. Stimuli–responsive liposomes are active delivery vesicles which will be changed via an external signal and release their loaded agents in the site ofaction. Many stimuli-sensitive liposomes have been and are being developed that avoid degradation of loaded drugs and release their containing substance in one single burst as a result of destabilization of the liposome membrane caused by certain internal or external stimuli (such as changes of physiological pH, tissue specific enzymes, physiological temperature or electrolyte concentration,etc.).

2. PH sensitive liposomes

Controlled drug release using liposomal targeting results in the accumulation of the drug at the site of action. The concept of pH-sensitive liposomes arose from the fact that certain enveloped viruses develop strategies to take advantage of the acidification of the endosomal lumen to infectcells. Accordingly, it was found that some pathological tissues such as tumors, inflamed and infected areas exhibit an acidic environment as compared to normal tissues.

Therefore, specific conditions found in the target tissues can be employed as a trigger for controlled release by the incorporation of pH-sensitive components into the liposomal bilayer. pH-sensitive liposomes are commonly stable at physiological pH; however, they undergo destabilization under acidic conditions leading to the release of their aqueous-loaded drugs and effective delivery of drug or gene fragments into the cytoplasm via the endocytic pathway.

Different types of macromolecules which formed pH-sensitivity in liposomes were used in pH- sensitive liposomes.

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- Polymorphic lipids –

The typical polymorphic lipid used to form pH-sensitivity in pH-sensitive liposomes is the unsaturated phosphatidylethanolamine, including dioleoyl phosphatidyl ethanolamine (DOPE), palmitoyl phosphatidylethanolamine and diacetyl nic-phosphatidyl-ethanolamine. To formulate pH-sensitive liposomes, DOPE is commonly used with mild acidic amphiphiles that act as stabilizers at neutral pH, such as oleic acid, cholesteryl hemisuccinate and palmitoyl homocysteine.

- Cage lipid derivatives

This kind of liposome that contains the derivatives of phosphatidylethanolamine or annular lipid compositions with alkyl ether, such as N-citraconic-dioleoyl-phosphatidyl- ethanolamine, Ncitraconyl-dioleoyl-phosphatidylserine and poly(ethylene-glycol)-N- distearolyphosphatidyl-ethanolamine can reversibly display the ability to form non-bilayer phase simply with the drug-permeable membranes or with the fusion competent.This can destabilize the stability of the biofilm and thus increase the permeability of entrapped drugs.

- Synthetic fusogenic peptides and proteins

In this type of liposome, the peptide or protein is inactive when such liposomes are in the neutral pH environment. However, in the acidic environment, the conformation of the fusion peptide or protein is changed and the fusion between liposomal and cell membrane is stimulated and consequently, the pHsensitive liposomes release the encapsulated drugs eventually.

- Ph sensitive polymers

These polymers showed interesting features in the release of drugs upon an external stimulation and can interact with the lipidbilayer, which promotes the fusion between liposomes and endosomal membrane.

3. Thermo sensitive liposomes

A potential form of targeted drug delivery, which currently attracts enhanced interest, involves the use of thermo-sensitive liposomes in some diseases like cancer chemotherapy. The idea of using temperature sensitive nanocarriers naturally came from the fact that many pathological areas demonstrate distinct hyperthermia. Furthermore, there are various means to heat the required area in the body. The therapeutic effects of liposomal formulations can be also enhanced by receptor targeting and by triggering drug release within the tumor, by decomposition of the formulation at increased temperature. Hence, targeted temperature sensitive liposomes have attracted much attention and are considered to be a promising tool to achieve site specific delivery of drugs.

Thermo-sensitive liposomes were first formulated by Yatvin et al. These liposomes have been prepared using lipids whose membranes undergo a gel-to-liquid crystalline phase transition a few degrees above physiological temperature. A combination of thermo-sensitive liposomes with mild hyperthermia has demonstrated better therapeutic efficacy than simple liposomal chemotherapy, by increasing the intra-tumoral drug concentration. A temperature-sensitive folate targeted doxorubicin-containing magnetic liposome also been developed by Pradhan et al. for thermochemotherapy of cancer.

4. PEGylated stealth liposomes

Nanoparticles and other macromolecules introduced into the bloodstream are distributed throughout the body via the vascular system. Clearance of these species is mediated by the renal system or the mononuclear phagocytic system (MPS), also known as RES. Nanoparticles up to 8 nm are cleared rapidly by the kidneys with minimal catabolism. Particles larger than 8 nm evade the renal system typically and are cleared by the MPS, which represents the primary means of nanoparticle clearance from blood circulation. Shielding nanoparticles from opsonization, known as stealth, is a critical feature in nanoparticle design.

Stealth liposomes can be formed by introducing coating nanoparticles with hydrophilic polymers to escape from MPS detection. In intravenous administration, for reaching to the target tissue or cells and organizing cytoplasmic delivery, it is necessary for liposomes to be stable in biological fluids and display long circulation times. PEGylation inhibits liposome aggregation and non- specific interactions by altering the physicochemical properties of liposomes (particularly surface charge and hydration). In addition, the PEG polymers also create a physical barrier to opsonins and other serum proteins, which prevent their adsorption to the liposomes surface and the subsequent liposomes clearance.

5. Immunoliposomes

The use of antibodies attached to the surface of liposomes to form immunoliposomes,

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where they are selectively adsorbed to a chosen antigenic site, is now a well-established approach to liposome targeting. The first report on antibody-targeted liposomes came from Torchilin et al. three decades ago. These antibody targeted liposomes were shown to be able to specifically bind to the antigen that is expressed on the target cells. In contrast to many liposome studies which have concentrated on targeting rather than actual drug delivery, immunoliposomes bearing antibodies have been used to investigate the delivery of several anticancer agents such as Adriamycin and Daunomycin. There are many challenges to translating active targeted liposomes into routine clinical use.

The targeting and retention of drug-carrying immunoliposomes to specific antigenic sites must be followed by the release of the drug. This can occur by passive diffusion through the liposomal bilayer, but this may be too slow to maintain an effectively high drug concentration in the vicinity of the cell membrane.Active targeting using immunoliposomes has several advantages over antibodies–drug conjugates. Immunoliposomes can carry a significantly larger number of drug molecules compared with simple conjugates and also can encapsulate drugs with different physicochemical properties. Drugs encapsulated in immunoliposomes can also reach their intracellular target by diffusion after release from immunoliposomes associated with target tissue. Therefore, unlike antibodies–drug conjugates, in somecases immunoliposomes do not have to undergo receptor–mediated endocytosis to deliver their contents intracellularly.

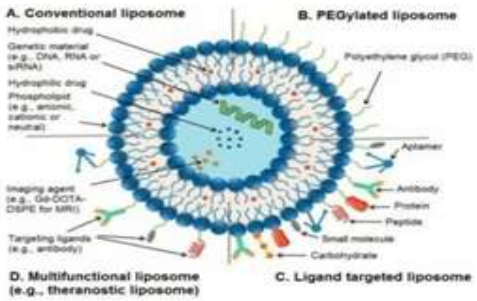
6. Cationic liposomes

Cationic liposomes are usually employed as a gene delivery system due to their low toxicity and immunogenicity, potential for oncogenicity, size independent delivery of nucleic acids as well as ease of preparation and quality control. Lipid base membranes are mainly composed of a combination of natural or synthetic phospholipids as well as cholesterol, but additional lipids carrying neutral, cationic or anionic groups are often involved. Cationic lipids are composed of a charged headgroup, consisting of a hydrophobic moiety and a linker (spacer, backbone) where this linker acts as a connector between hydrophobic domain and cationic head. The type of the spacer chain with regard to chain length, saturation and symmetry has an impact on membrane fluidity and thereby the transfection effi-ciency of the cationic liposomal systems. Depending on the positive (cationic lipid) to negative (phosphate group on nucleic acid) charge ratio, lipoplexes may enter the cells through electrostatic interaction with such charged residues at the cell surface as sialic acid moieties, or by hydrophobic interaction with the hydrophobic areas of the plasma membrane.

7. Fusogenic liposomes

The therapeutic application of many anticancer drugs is limited, or not effective, because of their poor cellular uptake, lack of specificity toward tumor tissues and the ability of cancer cells to develop resistance to chemotherapeutic agents. To this point, multiple attempts have been made to develop a tumor-targeted pharmaceutical carrier with the ability to provide an effective cellular internalization of an anticancer drug directly into the cytoplasm by passing the endocytic pathways (with protecting drugs from lysosomal degradation), thus enhancing the drug efficacy. The interactions between the liposomal vesicles and cells can occur via one or more processes including stable physical adsorption, endocytosis, lipid exchange and fusion. A number ofdifferent approaches have been used to create phospholipid.liposomes that have the capacity to fuse with cellular membranes. In some fusogenic liposomes, fusogenicity comes from membrane-associated proteins or peptides. In other types, it depends on specific interactions between the liposomes and target membrane.receptors. Furthermore, liposomes containing negatively charged phospholipids become fusogenic in the presence of calcium.

Different types of liposomal drug delivery system:- 8



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(A) Conventional liposome -

Conventional liposomes were the first generation of liposomes to be developed. Liposomes consist of a lipid bilayer that can be

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composed of cationic, anionic, or neutral (phospho)lipidsand cholesterol, which encloses an aqueous core. Both the lipid bilayer and the aqueous space can incorporate hydrophobic or hydrophilic compounds, respectively. Conventional liposomal formulations reduced the toxicity of compounds in vivo, through modifying pharmacokinetics and biodistribution to enhance drug delivery to diseased tissue in comparison to free drug. However, the delivery system was prone to rapid elimination from the bloodstream, therefore limiting its therapeutic efficacy.

(B) PEGylated liposome -

Liposome characteristics and behavior in vivo can be modified by addition of a hydrophilic polymer coating, polyethylene glycol (PEG), to the liposome surface to confer steric stabilization. The hydrophilic polymer, polyethylene glycol (PEG), has been shown to be the optimal choice for obtaining sterically-stabilized liposomes. This not only reduces the elimination of drugs by prolonging blood circulation and providing accumulation at pathological sites, but also attenuates side effects.

(C) Ligand-targeted liposome—

Liposomes can be used for specific targeting by attaching ligands (e.g., antibodies, peptides, and carbohydrates) to its surface or to the terminal end of the attached PEG chains.Ligand-targeted liposomes offer a vast pLigand-targeted liposomes offer a vast potential for site-specific deliveryof drugs to designated cell types or organs in vivo, which selectively express or over-express specific ligands (e.g., receptors or cell adhesion molecules) at the site of disease . Many types of ligands are available, such as antibodies, peptides/proteins and carbohydrates.

(D) Theranostic liposome—

A single system consists of a nanoparticle, a targeting element, an imaging component, and a therapeutic component. Overall as a drug delivery platform, liposomes offer a dynamic and adaptable technology for enhancing the systemic efficacy of therapeutics in various diseases.

Kaposi's Sarcoma, One Instance of Successful Liposomal Drugs Applications:-

Kaposi's sarcoma is a progressive multifocal anti-proliferative cancer primarily known as endometrial sarcoma. This cancer is more common in HIV patients whose immune system is weakened. Furthermore, it has been commonly seen in skin tissue and may also involve other tissues. Hence, this disorder is generally referred to as skin mucosal sarcoma .

To treat this disease, modified long-circulating liposomes can be helpful. In this regard, liposomes passively target tumor cells. Moreover, the effect of EPR and specific binding increases the concentration of the therapeutic drug in cancer tissues 5 to 11 times higher than normal skin . For this purpose, Doxorubicin is used for the treatment of this disease. Correspondingly, entrapment of the doxorubicin into liposomes (which was PEGylated to prolong its halflife) prevents normal tissues from being exposed to the drugs. It also reduces drug uptake by these healthy doxorubicin-sensitive tissues such as the heart .

Additionally, the liposomal form of doxorubicin, Doxil, is a type of anthracycline drug which is approved for clinical administration by US-FDA. It is used to treat AIDSrelated Kaposi sarcoma and multiple myeloma . Doxil has better therapeutic efficacy and less toxicity than free doxorubicin, which can be attributed to its ability to target tumors indirectly. It is also passive targeting due to leakage of tumor vessels and the EPR effect . Moreover, the Doxil unilamellar liposomes are<100 nm in size and have been used to treat various cancer types . Analyses have also proved that free doxorubicin concentration is lower than that of Doxil at the target tissue site. In this regard, Ogawara et al. investigated the effect of Doxil (formed by binding doxorubicin toPEG liposomes) on cancer cells in male mice and showed that PEG liposomal doxorubicin or Doxil1 had been effective on both doxorubicin-resistant and doxorubicin-sensitive C26 cell groups .

This can highlight the significance of the exploitation of liposomal NPs. Because they can be consumed to overcome the resistance of cancer cells to common chemotherapy agents at low costs without time-consuming research works to discover new clinical therapeutic compounds . The application of nanoparticles, such as liposomes, to deliver doxorubicin to tumor tissues have been widely investigated. Entrapment of ATP-binding cassette transporter superfamily B member 1 (ABCB1) substrate doxorubicin into liposomes can increase drug uptake and enhance its intracellular distribution within cancer cells, especially ABCB1-expressing
cancer cells.

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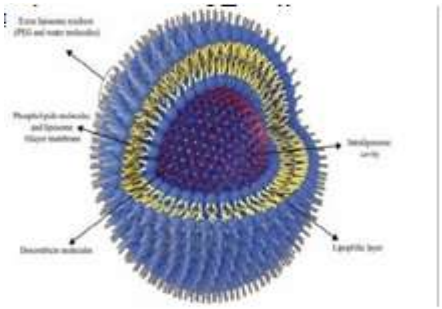
Simple structure of Doxil



Although the application of liposomal NPs to treat cancer has been touted as a viable solution for drug delivery and affecting tumor cells, drug delivery to cancerous tissues in the central nervous system (CNS) has remained a significant challenge. In addition, drug delivery to central nervous system cells faces many turbulences owing to a blood–brain barrier (BBB). However, thisproblem can be partially solved by developing new methods and using lipid-based compounds.

BIOLOGICAL CHALLENGES FUSING LIPOSOMAL DRUGS DELIVERY SYSTEM:-13,14

As with any foreign particle that enters the body, liposomes encounter multiple defense systems aimed at recognition, neutralization, and elimination of invading substances. These defenses include the RES,opsonization, and immunogenicity.

The Reticuloendothe lial System(RES) and Liposome Clearance	The RES is the main site of liposome accumulation following their systemicadministration. Primary organs associated with the RES include the liver, spleen, kidney, lungs, bone marrow, and lymph nodes. The liver exhibits the largest capacity for liposomal uptake followed by the spleen, which can accumulate liposomes up to 10-fold higher than other RES organs. 1. The ability of the RES to sequester liposomes from the circulation isattributed to fenestrations in their microvasculature. 2. Liposomes are cleared in the RES by resident macrophages via direct interactions with the phagocytic cells. 3. The cells of the RES are also part of the innate immune system, which has raised the question of whether macrophage saturation by liposomes leads to immunosuppression and increases the risk of infections. Solution :- Anti-cancer liposomes that contain cytotoxic drugs, which are capable of inducing macrophage destruction. But there have been indirect signs that suggest the possibility of some immune suppression, dose- dependent clearance saturation effect due to partial blockade of the RES in the liver.

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Opsonins and Vesicle Destabilization	The degree of interaction between liposomal drug delivery systems and plasma proteins is important in determining overall nanocarrier biodistribution, efficacy, and toxicity. Plasma proteins have been shown to play a pivotal role in liposomal clearance by the RES via opsonization, as well as in vesicular destabilization. Opsonization of liposomes by serum proteins depends on a variety of factors including size, surface charge and stability. 1. The extent of this interaction has been shown to decrease with liposome size from 800 to 200 nm in diameter, as small liposomes cannot support opsonic activity. Solution:- the presence of high electrostatic charge can still promote the interaction of liposomes with biomolecules that can serve as opsonins. Incorporation of cholesterol into the liposomal membrane abates lipid exchange with other circulating structures (e.g red blood cells and lipoproteins) that can cause the depletion of high phase transition temperature lipids and their replacement with less physiologically stable components.

The Enhanced Permeability and Retention (EPR)Effect	The EPR effect refers to the increased permeability of the vasculature that supplies pathological tissues (e.g., tumors and conditions involving inflammation). At these sites, deregulations in angiogenesis and/or the increased expression and activation of vascular permeability factors predominates, which leads to fenestrations that can range from 300 to 4700 nm. This allows liposomes to extravasate and accumulate by passive targeting. However exposure to inflammatory mediators increases permeability of the microvasculature, with the formation of gaps of up to 1 μm. Solution :- all types of liposomal delivery systems are subjected to the EPR effect, with PEGylated liposomes having an advantage due to having reduced RES clearance and extended circulation time.

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The Accelerated Blood Clearance (ABC) Phenomenon	The interaction of liposome components with the immune system has contributed to the challenges in translation to clinical use. Synthetic modifications to enhance their utility as drug delivery vehicles can result in antibody production against their various components and/or the encapsulated cargo. For example, repeated injection of PEGylated liposomes has been associated with loss of their long circulating properties and subsequent clearance from the blood, this phenomenon is known as the ―accelerated blood clearance‖ (ABC) phenomenon. Solution:- Increasing the phospholipid dose has been suggested to cause PEG-reactive B cells to become apoptotic, reducing anti-PEG IgM production and thus abating the ABC phenomenon. Generally higher doses (15 μmol phospholipid/kg) are administered clinically, which may account for this absence of the ABC phenomenon, this response may also be due to doxorubicin-mediated macrophage death and the inhibition of B-cell proliferation and/or the death of proliferated B-cells .

Complement Activation– Related Pseudoallergy (CARPA)	Some liposomal systems are able to trigger the innate immune response, with subsequent activation of the complement system to trigger an acute hypersensitivity syndrome known as complement activation–related pseudoallergy (CARPA). A relatively high percentage of patients (2–45%) have been reported to develop infusion-related hypersensitivity reactions to liposomal drug therapy. In addition, CARPA has been reported with both experimental and clinically approved liposomal formulations (e.g., Doxil®, Ambisome, and DaunoXome®). CARPA is an immediate, non-IgE- mediated hypersensitivity reaction that involves symptoms such as anaphylaxis, facial flushing, facial swelling, headache, chills, and cardiopulmonary distress. Solution :- The sensitivity of different species to liposomal CARPA shows substantial variation, with some species (dogs and pigs) also showing tachyphylaxis (tolerance induction) following additional doses ,desensitization protocols using empty liposomes may be used to prevent CARPA, as well as pre-administration of complement inhibitors (e.g., soluble C receptor type 1, anti-C5 antibody, and indomethacin).

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Liposomal drugs nanoparticles officially approved for various cancertreatment :- 11

Product commercialname	Active therapeuticagent	Liposomal nanoparticle platform	Indication
AmbiSome	Amphotericin B	PEGylated liposome	Aspergillosis Cryptococcosis candidiasis
DepoDur	Morphine sulphate	Extended-ReleaseLiposome	General pain reduction
Inflexal	Inactivated hemagglutinin of influenza virus strains A and B	Liposomes	Prevention of influenza disease
DaunoXome	Daunorubicin citrate	Conventionalliposome	Blood cancer HIV-related Kaposisarcoma
Depocyt	Cytarabine	Conventionalliposome	Acute myeloid leukemia (AML), acute lymphocytic leukemia
Doxil	Doxorubicin	PEGylated liposome	Ovarian and breast cancer, HIV-related Kaposi sarcoma, multiple myeloma
Epaxal	Inactivated hepatitis A virus (strainRG- SB)	Liposomes	Prevention of Hepatitis A virus
Visudyne	Verteporfin	Conventionalliposome	PDT sensitizer,pathologic myopia, ocular histoplasmosis

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Mepact	Muramyl tripeptide phosphatidylethanolamine	Conventionalliposome	Solid tumors chemotherapy
Nyotran	Nystatin	Conventionalliposome	Systemic fungal infections
Onivyde or MM-398	Irinotecan	PEGylated liposome	Post-gemcitabine metastatic Pancreatic cancer
Marqibo	Vincristine	PEGylated liposome/ conventional liposome	Philadelphia chromosome-negativ e acute lymphoblastic leukemia

II. CONCLUSION:-

Drug delivery systems are designed to stably and efficiently carry anticancer agents to tumor sites. Liposomes have attractive properties include biocompatibility, low toxicity, lower clearance rates, the ability to target specific tissues and controlled release of drugs. They offer numerous advantages over conventional chemotherapy using free drug treatment. Liposomal drugs have high encapsulation capacity, hence shows a significant anticancer activity with decreased toxicity preferentially cardiotoxicity. Liposome-based drug delivery systems are able to modify the pharmacokinetics and pharmacodynamics of cytostatic agents, enabling us to increase the concentration of the drug released into the neoplastic tissue and, at the same time, reducing the exposure of normal tissue to the drug. Some of the most successful delivery methods rely on PEG conjugated lipids. In fact, the first FDA approved nano-drug, doxorubicin, is delivered using PEGylated liposomes. When doxorubicin is incorporated in PEGylated liposomes, it minimizes the uptake and clearance by the RES, which prolongs the serum and plasma half-life. Advantages of marketed liposomal drugs include a reduced toxicity by increased vascular permeability/accumulation at the target tissue and an ability to encapsulate drugs of different lipophilicities while protecting them from biodegradation.

REFERENCE:-

[1]. Harshal R. Pawar, Sagar S. Bhosale, Nikita D. Derle*,Use of liposomes in cancer therapy, Int. J. Of Pharma. Sci. And Res., 2012,vol.3 ,issu 10, p. 3585-3590

[2]. Akulapalli Sudhakar, History of Cancer, Ancient and Modern Treatment Methods, J Cancer Sci Ther. 2009 December 1; 1(2): 1–4. doi:10.4172/1948-5956.100000e2.

[3]. 3 Juan Lao,Julia Madani,Teresa Puértolas,María Álvarez,Alba Hernández, Roberto Pazo-Cid, Ángel Artal,and Antonio Antón Torres, Liposomal Doxorubicin in the Treatment of Breast Cancer, J. of Drug Delivery Vol. 2013, Article ID 456409, 12 pages, http://dx.doi.org/10.1155/2013/456409 .

[4]. Jaspreet K. Vasir, M.S.Vinod Labhasetwar, Targeted Drug Delivery in Cancer Therapy, Tech. in Cancer Res. & Treatment ISSN 1533-0346Volume 4, Number 4, August (2005). www.tcrt.org.

[5]. Dena Tila, Saeed Ghasemi, Seyedeh Narjes Yazdani-Arazi and Saeed Ghanbarzadeh, Functional liposomes in the cancer-targeted drug delivery, J. of Biomaterials Applications, DOI: 10.1177/0885328215578111.

[6]. Preeti Kumari, Balaram Ghosh, and Swati Biswas, Nanocarriers for cancer-targeted drug delivery, Journal of Drug Targeting 2015, . DOI: 10.3109/1061186X.2015.1051049.
[7]. Yogeshkumar Malam, Marilena Loizidou and Alexander M. Seifalian, Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer, Trends in Pharmacological Sciences.
[8]. Temidayo O. B. Olusanya 1 ID , Rita Rushdi Haj Ahmad , Daniel M. Ibegbu , James R. Smith and Amal Ali Elkordy, Liposomal Drug Delivery Systems and Anticancer Drugs, Molecules 2018, 23, 907; doi:10.3390/molecules23040907.
[9]. Oula Penate Medina, Ying Zhu and Kalevi Kairemo, Targeted Liposomal Drug Delivery in Cancer, Current Pharmaceutical Design, 2004, Vol. 10, No. 24, p. 2981-2989.
[10]. Vladimir P. Torchilin, RECENT ADVANCES WITH LIPOSOMES AS PHARMACEUTICALCARRIERS,Drugs discovery volume 4 FEBRUARY 2005,p. 145-159, www.nature.com/reviews/drugdisc
[11]. Foad Rommasi and Neda Esfandiari , Liposomal Nanomedicine: Applications for Drug Delivery in Cancer Therapy, Nanoscale Res Lett (2021) 16:95, https://doi.org/10.1186/s11671- 021-03553-8
[12]. Robert A. Weinberg , How Cancer Arises, Scientific American September 1996, p.62-70 .
[13]. Campbell, R. B., Ying, B., Kuesters, G. M., and Hemphill, R. (2009). Fighting cancer: from the bench to bedside using second generation cationic liposomal therapeutics. J. Pharm. Sci. 98, 411–429. doi: 10.1002/jps.21458.
[14]. Lisa sarcoma, Tejaswi veerati, Fatimah Moheimani, Sherry Y., Anil k. Sood . Advances and Challenges of Liposome Assisted Drug Delivery. Front. Pharmacol., 01 December 2015 | https://doi.org/10.3389/fphar.2015.00286.