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Gene therapy of cancer

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ABSTRACT

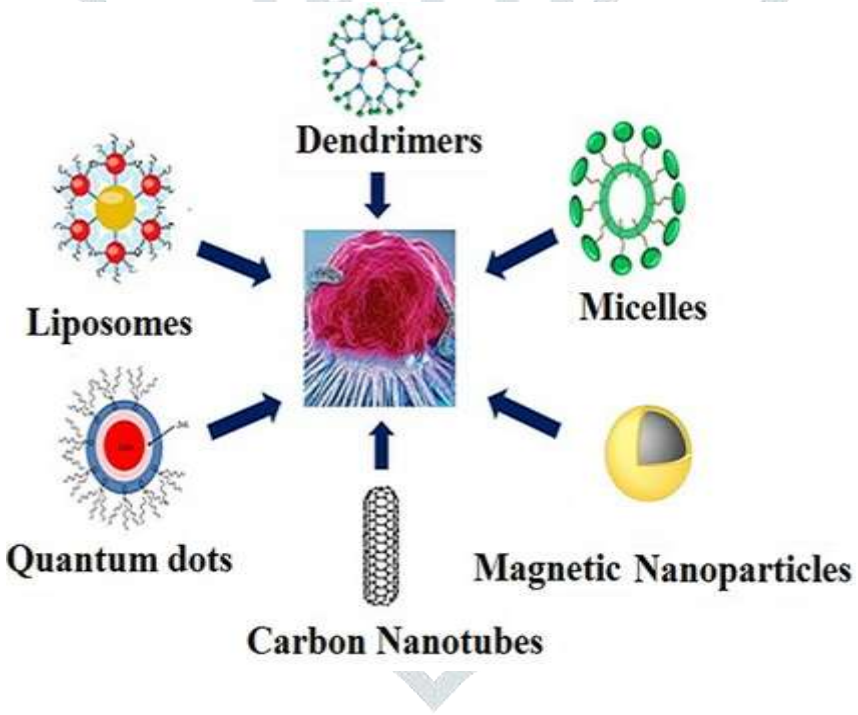
Cancer is a life-threatening disease contributing to ~3.4 million deaths worldwide. There arevarious causes of cancer, such as smoking, being overweight or obese, intake of processed meat, radiation, family history, stress, environmental factors, and chance. The first-line treatment of cancer is the surgical removal of solid tumours, radiation therapy, and chemotherapy. The systemic administration of the free drug is considered to be the main clinical failure of chemotherapy in cancer treatment, as limited drug concentration reaches the tumour site.

Most of the active pharmaceutical ingredients (APIs) used in chemotherapy are highly cytotoxic to both cancer and normal cells. Accordingly, targeting the tumour vasculatures is essential for tumour treatment. In this context, encapsulation of anti-cancer drugs within the liposomal system offers secure platforms for the targeted delivery of anti-cancer drugs for the treatment of cancer. This, in turn, can be helpful for reducing the cytotoxic side effects of anti-cancer drugs on normal cells. This short-review focuses on the use of liposomes in anti-cancer drug delivery.

Introduction

There is a high demand for advanced delivery systems that are suitable for the delivery of various active pharmaceutical ingredients (APIs), especially systems with low costs, high efficiency, low risks, and toxicity. Several APIs can be utilised better by employing nano-size drug delivery systems (DDS) that are designed to enhance the delivery of APIs with poor pharmacokinetics and biodistribution . For instance, most of the chemotherapeutic medications are characterised by poor pharmacokinetic profiles in addition to non-specific distribution in the body tissues and organs, causing serious side effects and systemic toxicity. Accordingly, nano-size structures-based pharmaceutical formulations (e.g., liposomes, polymeric nanoparticles, electrosprayed particles, and nanosuspension have demonstrated better therapy of the APIs. Moreover, due to the complexity of solid tumours, an effective penetration of anti-cancer agents encapsulated within a nanocarrier is the main challenge in cancer therapy.

Liposomes are the most commonly investigated nanostructures used in advanced drug delivery, which were first discovered by Alee Bangham in 1963. Liposomes are artificially spherical vesicles prepared from naturally-derived phospholipid. They entail one or more lipid bilayers with discrete aqueous spaces. They are well established for a range of pharmaceutical and biomedical applications with the unique capability of entrapment of both hydrophilic (polar) and hydrophobic (nonpolar) compounds due to their amphipathic nature in aqueous media. For instance, hydrophobic compounds entrap in the bilayer membrane, while hydrophilic compounds encapsulate in the aqueous core. Liposomes serve as DDSs due to their versatile structure; biocompatibility; and the fact they are naturally nontoxic, non-immunogenic, and biodegradable. Liposomes have several advantages contributing to drug delivery. They have a role enhancing drug solubility, serving as a sustained release system, providing targeted drug delivery , reducing the toxic effect of drugs, providing protection against drug degradation , enhancing circulation half-life of APIs], being effective in overcoming multidrug resistance , improving the therapeutic index of the entrapped drug, and protecting APIs against their surrounding environment



Advance drug delivery systems in anticancer

❖ Liposomes

Cancer is a life-threatening illness that leads to irregular and uncontainable growth of malignant cells. These uncontrolable cells can invade normal tissues and organs, causing undesirable growth and reactions that end up destroying them. Cancer is responsible for ~3.4 million deaths worldwide. Some of the well-known causes for cancer disease are smoking (causing lung) breast , and ovarian cancer , being overweight or obese (associated with 13 types

of cancer disease, such as breast cancer, kidney, womb and bowel cancers), intake of processed meat , radiation (causes skin cancer), family history, stress, environmental factors, and chance

Cancer cells are able to spread around the human body through blood vessels and lymphatic streams, causing metastasis by forming a secondary tumour . Anticancer agents are typically administered to the patients to kill cancer cells. These drugs work in two ways: by killing the cancer cells through direct exposure to the chemical agent and by inducing apoptosis (suicide of cancer cells).

Tumour vasculature is vital to preserving the tumour and aiding its growth. It is characterised by special physiological properties, being highly chaotic, complex, and porous in nature. Pore size ranges between 100–780 nm, while normal tissue junctions are <6 nm .The distance between tumour cells should be within a certain limit of the perfused blood vessels in order to obtain the required quantity of oxygen and nutrients to survive and proliferate . Accordingly, tumours can undergo extensive angiogenesis (the growth of new tumour blood vessels), grow beyond 1–2 mm in diameter, and form hypervasculatures, which can be defective and impair the lymphatic drainage systems. In addition to these properties, tumours canproduce three known vascular-permeability influences such as bradykinin, nitric oxide, and peroxynitrite. Targeting the tumour vasculature is essential for tumour treatment. This can be done by disturbing the angiogenesis using antiangiogenic agents (eg, Axitinib (Inlyta®), Bevacizumab (Avastin®) and Cabozantinib (Cometriq) or shutting down theexisting tumour blood flow, resulting in ischemia and tumour cell necrosis using vascular targeting agents (eg, 5,6-dimethylxanthenone-4-acetic acid (flavonoid derivative) andCombretastatin A-4 phosphate (tubulin binding drug). Accordingly, there isa high demand for

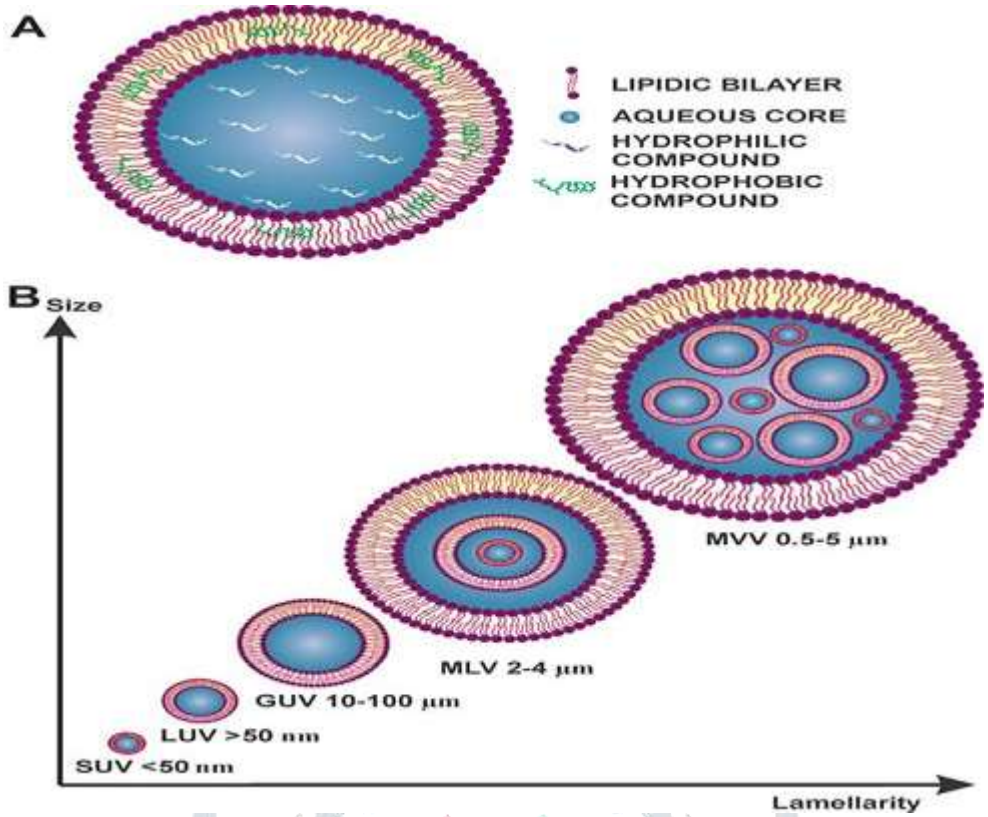
finding a proper controlled anticancer dosage to control this disease, as it is expected to be responsible for 13.2 million death cases by 2030.

Liposomal drug formulations offer the possibility of increasing efficacy while reducing the toxic side effects of chemotherapeutic drugs. They can also impact the pharmacokinetics and tissue distribution of the incorporated anticancer compound. They have been described as alternative DDSs that have been used to enhance the therapeutic index and significantly reduce the toxic effect of anticancer agents on normal tissues, including doxorubicin.

➢ Categories of liposomes

Numerous factors define liposomes properties such as the lipid composition, number of lipid bilayers, size, surface charge, and the method of preparation. Liposomal vesicles vary in size between 0.025 cm to 2.5 cm. They can be categorised according to the number of their layers (also Molecules referred to as lamellae): unilamellar (consisting of single phospholipid bilayer) or multilamellar (consisting of more than one unilamellar separated by layers of water (>500 nm)). Unilamellar vesicles are subdivided into small unilamellar vesicles (20–100 nm) and large unilamellar vesicles (>100 nm). Both the size and the number of lamellae in the liposomal structure are considered to be the most crucial factors affecting the vesicles half-life and the quantity of API that is to be encapsulated. This unique and flexible variety in the

liposomal structure distinguishes liposomes as the preferred carriers for a broad spectrum of therapeutic agents.



➢ Stability of Liposomes

Physical and chemical stability of the liposomes in terms of size distribution, entrapment efficiency, and minimal degradation of liposomal apparatuses is the major limiting step for drug delivery using this system. Chemical degradation of liposomes mainly occurs at the phospholipid bilayers level, in which two different reactions might develop: (i) hydrolysis of the ester bonds between fatty acids and glycerol backbone, and (ii) peroxidation of any available unsaturated acyl chain. These two reactions might lead to the development of short-chain lipids; subsequently, soluble derivatives will appear in the membrane that would significantly reduce the quality and stability of the liposomal system. With respect to physical instability, liposomes might undergo aggregation/flocculation and fusion/coalescence, which can ultimately change vesicle size and lead to significant loss of the encapsulated API.

Aqueous dispersions of liposomes suffer from elevated levels of instability due to the leakage of the encapsulated drug out from the phospholipid bilayers. In addition to this, aggregation of liposomes upon storage for a period exceeding the first few months of preparation was reported. Accordingly, it is more advisable to store liposomal preparations in solid form. Several methods

are available that show extendable techniques to stabilise the liposomal formulations, such as lyophilization, spray drying, and supercritical fluid.

Several factors that have an influence on liposomal system stability, such as liposomal composition (e.g., phospholipids-lipids with high phase transition temperatures), fatty acid

side-chains, polar head chemistry, chain length, and the degree of unsaturation, are preferred to maintain liposomal rigidity and phospholipid:cholesterol molar ratio (crucial for the liposomal stability and controlling drug release). Briuglia et al. (2015) demonstrated that 70:30 molar ratio of phospholipids (using 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), and distearoyl phosphatidylcholine (DSPC)): cholesterol achieved a liposomal formulation that can guarantee the stability and control over drug release [40] and surface potential (high surface potentialis directly related to the liposomal physical stability, as it helps to reduce the rate of fusion and aggregation.

One of the reasons for liposomal aggregation is the vesicle–vesicle electrostatic effect between the vesicles. Repulsive interactions, which are at least equal to the degree and range of the van der Waals force, are an essential requirement for stable liposomal formulatio. The physical stability of liposomes improves by increasing the surface charge density and reducing the ionic strength of liposomes (increases the electrostatic repulsive energies), especially when phosphatidylcholine and phosphatidylserine are used. However, scientists need to consider all influencing factors affecting liposomal stability and work around all of them. This is due to the fact these factors might be affected by certain factors that can disrupt the system. For instance, electrostatic stabilisation is very sensitive to the surface charge (pH) and salt concentration of the liposomal suspension. Electrostatic stabilisation can be improved by combining it with the steric stabilisation (so called electrosteric stabilisation), which can be obtained by covering the surface of the liposomes with an adsorbed coat of long, bulky molecules (which, for example, keep the distance between the vesicles).

➢ Influence of Liposomal Composition in Drug Delivery

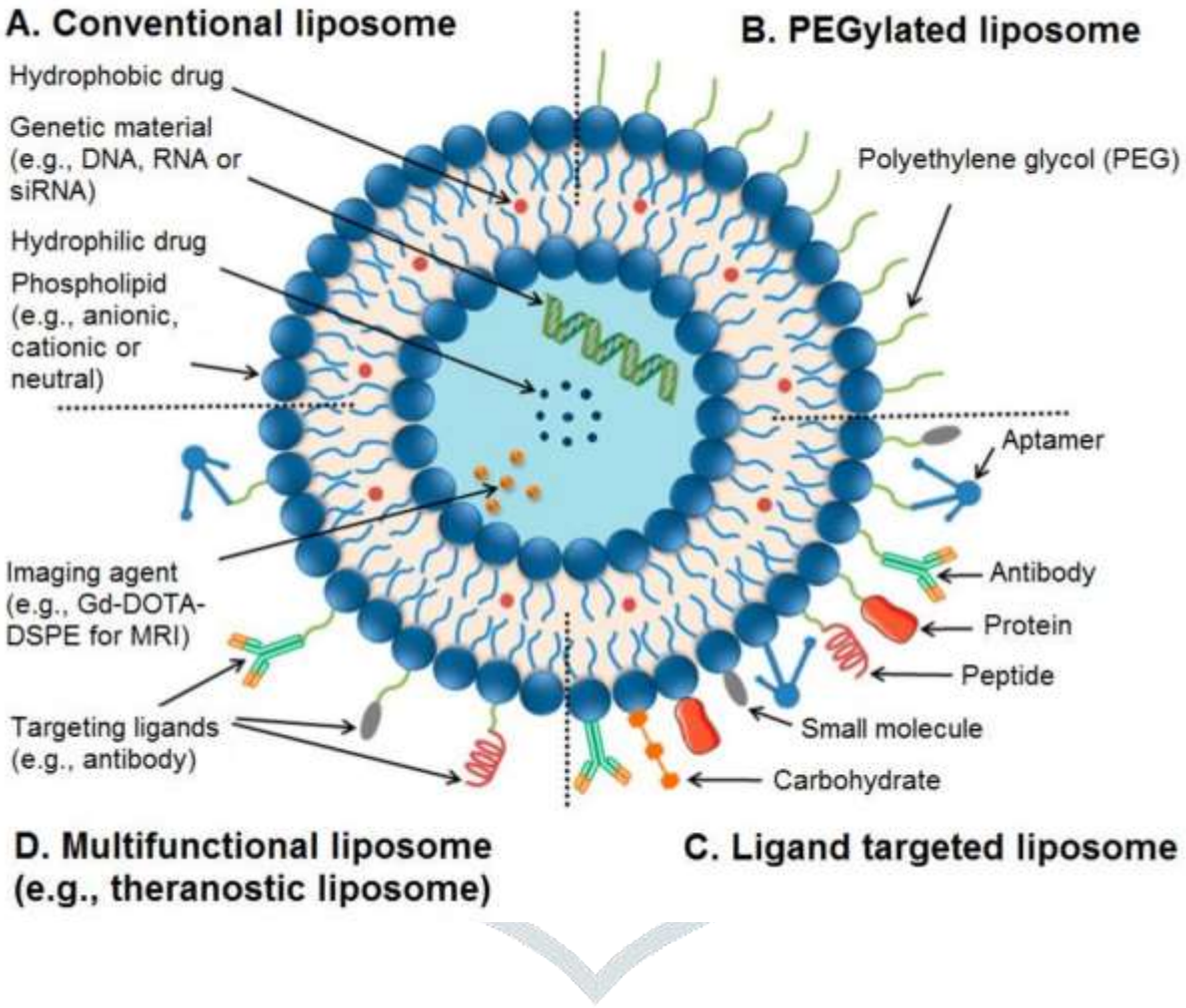
Phospholipids are the main building blocks of liposomes. These biomolecules are also the main components building the biological membranes. They are amphiphilic molecules that consist of a polar head (water soluble hydroxy groups) and insoluble backbone. Liposomes can be zwitterionic, positively or negatively charged, or uncharged. This is totally dependent on the polar head charge. There are two types of lipids currently utilised for liposome preparation: naturally occurring or synthesised double-chain lipids (consisting of phosphorus polar head and glycerol backbone) and sterols (e.g., cholesterol).

The most known lipids used in the liposomal formulations are phosphatidylcholine (zwitterionic), phosphatidylglycerol (negatively charged), phosphatidic acid, phosphatidylethanolamine (zwitterionic), and phosphatidylserine (negatively charged). Positively charged lipids (e.g., N-[1-(2,3-dioleyloxy)propyl]-N,N,N-triethylammonia (DOTMA) and 1,2-dioleoyl-3-trimethylammoniopropane (DOTAP)) are mainly used for gene delivery, as they interact with the negatively charged deoxyribonucleic acid (DNA and negatively charged APIs.

Cholesterol is another strategic component of liposomes. It has a modulatory effect on the properties of the lipid bilayer of the liposomes. It can control the stoutness in the liposome structure and increase the packing between the phospholipid molecules , resulting in more ordered conformation in the aliphatic tail region, reduced micropolarity, reduced bilayer flexibility to the surrounding molecules (especially water soluble molecules), and increases in the microviscosity of the bilayer. Cholesterol is also crucial for structural stability of liposomal

membranes against intestinal environmental stress. Cholesterol was found to influence liposomes size (increasing cholesterol concentration increases liposomes size in addition to shape transition), provide permeability and fluidity, and consequently modulate the release of hydrophilic compounds from liposomes.

It is also possible to use surface functionalisation of liposomes by a variety of agents to overcome the limitations of these nanocarriers in terms of biological and physiological barriers. For example, liposomes can be functionalised with polyethylene glycols (PEGs), aptamers, antibodies, proteins, peptides, ligands, carbohydrates, or small molecule



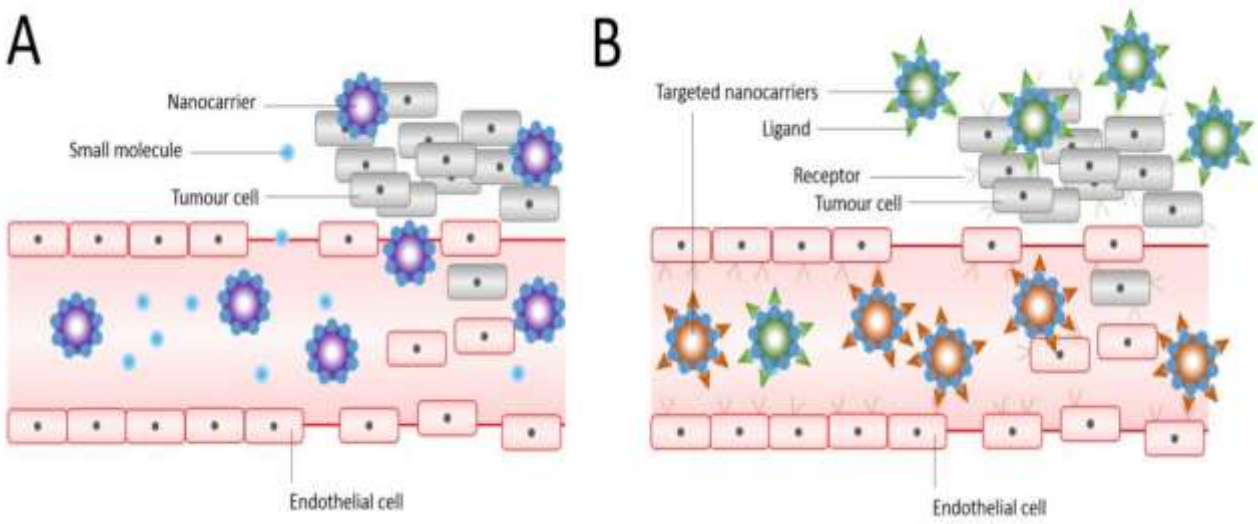
Structure of conventional and functionalised liposomes

➢ Liposomes as Targeted Drug Delivery System for Cancer Treatment

Advancements in liposomal vesicle development have achieved both controlled drug release and targeted drug delivery (disease-specific localisation). This property is essentially helpful for cancer treatment as surgical resection, radiation therapy, and chemotherapy are the first-line treatment of cancer. Some cancerous states require systemic administration of the chemotherapy. So far, 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 administrated directly into the blood stream that circulates the body. The chemotherapeutic agent can then be uptaken by cancer and normal tissues, leading to severe toxicity to different body organs, such as heart, kidneys, liver, and others. As a result, sometimes the highest possible dose of chemotherapy is administrated to the patients to maximise the quantity of the medication taken up by the cancer cells. The success of cancer treatment basically depends on its capability to decrease the size and remove tumours without affecting normal tissues, thus increasing patients' survival time and enhancing their quality of life

The encapsulation of chemotherapeutic agents within liposomal structures can limit the normal tissue uptake of the medication and thus improve its therapeutic index. By means of passiv targeting, liposomes can concentrate preferentially on the tumour (typically over 24–48 h) via the enhanced permeability and retention (EPR) effect of the vasculature, in which leaky tumour vessels unite with absent lymph drainage. In other words, passive targeting ofliposomes happens by transferring them into the tumour interstitium via leaky tumour vasculature through molecular.This can be done by adding certain antibodies to the liposomal surface—so called immune liposomes (ILP)—which are specific to the cancer cells or to the endothelial cells of the tumour vasculature. Maruyama et al. (1999) developed the pendant type ILP (34A-PEG-ILP),which is a long-circulating polyethylene glycol (PEG)-ILP attached to antibodies (34A antibody) at thedistal end of PEG chain. These ILPs showed high targetability to the site of action (lung endothelial cells and tumour tissue)—more than ordinary liposomes. This 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 tumour tissues or cells have a specific antigen for the targeted antibody to bind to. Accordingly, this approach is limited to the antigen-antibody specifications.



Passive (A) and (B) active targeting of nanocarriers

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 tumour tissue. This can be achieved by the effect of EPR or by releasing the agent within the tumour vasculature using liposomes particularly designed to
respond to a precise external trigger (e.g., 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 formulation 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 enhancing the permeability of the target cells to API that release from the liposomes, by enhancing the fusion or endocytosis effect of target cells to the directly transferred drug from liposomes, and by
improving drug release from liposomes by reducing the local pH of the target site of action. The first thermosensitive liposomes were developed by Yatvin et al. (1978), in which the formulation used the two lipids DPPC:DSPC (molar ratio of 3:1), encapsulating neomycin (an aminoglycoside antibiotic) as API. A local hyperthermia is then initiated (>40–42 _C), triggering drug release at the targeted site. The first animal test of an anticancer formulation using thermosensitive liposomes was tested by Weinstein et al. in 1979. A mouse with lung cancer was treated with methotrexate-encapsulated thermosensitive liposomes (DPPC:DSPC, 7:3 molar ratio). The results showed a 4-fold increase of the drug quantity that reached the tumour tissue. However, the elimination of the liposomes within 1 hour of administration was the major limitation of this formulation.

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 vesicles 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. Maintaining an optimum hyperthermal effect is another major limitation in clinical settings using thermosensitive liposomes, as it can result in overheating of tissues. Magnetic liposomes-mediated unit chemotherapy and hyperthermal effect was developed as a potential solution to overcome this problem. For instance, a novel magnetic liposomal formulation for self-controlled hyperthermia and chemotherapy was designed by Gogoi et al. (2017). Liposomes co-encapsulated with dextran-coated biphasic suspension of La0.75Sr0.25MnO3 (LSMO) and iron oxide nanoparticles were developed using paclitaxel (PCX) as a model drug. Evaluation of the therapeutic efficacy of the formulation showed a 2.5-fold (mean tumour volume 2356 _ 550 mm3) reduction of the tumour growth after a single administration of the drug-loaded magnetic liposomes. A 3.6-fold (mean tumour volume 1045 _ 440 mm3) reduction of the tumour growth after a double dose treatment was reported as compared to the growth reduction effect of the corresponding control (mean tumour volume 3782 _ 515 mm3). With no significant leaching of liposomes, biocompatibility and therapeutic evaluation studies demonstrated the potential use of magnetic liposomal formulation for the treatment of drug-resistant or physiologically vulnerable cancer. The combination of chemotherapy and thermotherapy was also reported using doxorubincin (DOX)-loaded magnetic liposomes (using citric acid-coated magnetic nanoparticles). About 130 nm-sized magnetic liposomes were developed utilizing hydrogenated soy phosphatidylcholine

(HSPC)/1,2-distearyl-sn-glycero-3 phosphoethanolamine (DSPE)/cholesterol (12.5:1:8.25 molar ratio) and DOX by rotary evaporation and ultrasonication process. In vitro cytotoxicity and hyperthermia studies evaluated against colorectal cancer revealed that the magnetic liposomes displayed no cytotoxicity, with approximately 56% tumour cells being killed. This study demonstrates the effectiveness of the combination of hyperthermia and chemotherapy treatment in one system as compared with individual treatment

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 (sPLA2) (raises in prostate , 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 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).

Conclusion

Oncology pharmacist face a constant challenge with patient who cannot swallow oral anticancer drug, making extemporaneous oral liquid preparation a requirement. Improper extemporaneous preparation of this agent, especially which the traditional chemotherapy with a narrow therapeutic index, may increase the risk of over- or under dosing. In community pharmacies, multiple barriers exits that prevents this pharmacies from preparing extemporaneous oral anticancer drug formulations for a patients use at home. In a home setting, patients or caregivers without proper counselling and education on how safely handle chemotherapy are at increased risk for exposure to these drugs.

Based on a review of the literature, compounding recipes are available for 46% of oral anticancer agents . A paucity of data exits on dose uniformity, bioequivalence and stability of extemporaneous oral liquid formulations of extemporaneous oral liquid formulations of anticancer drugs. Pharmacist must have an understanding of the basic scientific principle are an essential foundation for the proper preparation of the extemporaneous oral anticancer liquid formulation the collaborative effort of a multidisciplinary team can also help identify different barriers in the community setting especially in areas where community pharmacies may lack resources for the extemporaneous compounding of oral chemotherapy, and to find ways to coordinate better pharmaceutical care.

There are great opportunities for oncology pharmacist, as a resources educating and monitoring patients receiving oral chemotherapy to ensure dosing accuracy, safe-administration and proper disposal of hazardous drugs. Development of national guidelines to promote standards of practice in the community and / or home setting is urgently needed to help improve the safety of dispensing and handling oral chemotherapeutic agents, including extemporaneously compounded oral formulations of these drugs.

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