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Recent Trends in Nano Drug Delivery Systems to Treat Cancers: With Special Focus on Liposomal Drug Delivery Systems

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Abstract

Cancer treatment is an area of ongoing concern and requires a great deal of research in order to provide patients and providers with the best possible therapeutic options. Conventional chemotherapeutic agents typically cause serious, unexpected side effects from the harmful consequences of the agents on normal, noncancerous tissues. Drug delivery has also been a challenge as inefficient delivery has been shown to cause decreased drug effectiveness as well as adverse effects on tissues not targeted by the drug. Improving the therapeutic index of these agents by bettering the targeted drug delivery systems is an area of much research currently. Liposomes are nanoparticles capable of entrapping both hydrophobic and hydrophilic drugs, thus making them a prime candidate for potential targeted drug delivery systems in anticancer therapies. Previous studies have indicated that liposomal encapsulation protects chemotherapeutic agents from rapid degradation and reduces toxicity by preventing it from reaching systemic circulation. Compared to conventional methods, this form of nanotechnology holds promise in creating more effective targeted delivery as well as improving the therapeutic index. This could greatly improve patient outcomes and more available treatment options in the future and pave the way for researchers to better understand and implement this nanotechnology in real time.

Keywords: Nanotechnology; Nano drug delivery systems; Cancer treatment; Nanomaterials; Anticancer drugs; Liposomal drug delivery; Immunotherapy using liposomal delivery systems.

Introduction

Nanotechnology refers to the newly developing field in which therapeutic agents are adapted and applied at the nanoscale level. The systematic approach considers the individual molecules and interacting molecules at play in reference to the bulk macroscopic properties of a given material or device. In this case, nanotechnology has provided a new avenue of research in the market of cancer research in the form of liposomes. These nanoparticles have demonstrated great promise in cancer treatment, as a more effective approach to delivering necessary therapeutic and chemotherapeutic agents in treatment. The application of innovative nanotechnologies to medicine, otherwise known as nanomedicine, has the potential to significantly benefit clinical practice, offering solutions to many of the current limitations in diagnosis, treatment and management of human disease. The diverse branches of nanomedicine include tissue regeneration, drug delivery and imaging.

Liposomes

Liposomes are closed spherical vesicles consisting of a lipid bilayer that encapsulates an aqueous phase in which drugs can be stored. The liposome diameter varies from 400 nm to 2.5 mm [1]. Nanoparticles, which are particles ranging in size from 1 to 100 nm, exhibit unique physical and chemical properties that can be exploited for drug delivery by conjugation with drugs. Both these emerging nanoscale drug delivery systems can be used to improve current treatment regimens. High drug toxicity is a barrier to treatment because side effects limit the drug dosage that can be administered. This is best exemplified by cytotoxic cancer drugs. Although very effective *in vitro*, in human clinical use the drugs act indiscriminately on both cancerous and healthy tissues. Side effects can be both serious and unpleasant and range from nausea and hair loss to neuropathies, neutropenia, and kidney failure. Therefore, drug non-specificity

limits efficacy. The ideal nanoscale drug delivery system ensures that the conjugated or bound drug–carrier complex arrives and acts preferentially at the selected target. Targeting of the drug nanocarrier complex can be active, whereby the complex incorporates a ligand specific for the receptor or epitope ofthe target tissue. In passive targeting, complexes diffuse and accumulate at sites with excessively leaky microvasculature, such as tumors and inflamed tissues, with normal endothelium being much less permeable. Subsequent extravasation of complexes takes place either via transcytosis, whereby macromolecules are internalizedfrom the blood at points of invagination of the cell membrane, or paracellularly, via diffusion through the tightjunctions of endothelial cells [1]. Particularly in cancers, an imbalance in factors that regulate angiogenesis, such asoverexpression of vascular endothelial growth factor (VEGF), results in both increased vascular permeabilityand chaotic tumor-vessel architecture. In combination, these effects cause enhanced permeation and retention (EPR), resulting in high local drug concentrations [1].

Key properties of any nanomaterial

Kay properties of nanomaterials used in drug delivery are its biocompatibility and biodegradability, so that the unloaded carrier is degraded or metabolized into nontoxic components and cleared through the circulation. Materials are cleared according to size. Small particles (0-30 nm) are rapidly cleared by renal excretion. Nanocarriers greater than 30 nm are cleared by the mononuclear phagocyticsystem (MPS), consisting of macrophages located in the liver (Kupffer cells) and the spleen, which act as phagocytotic scavengers [1]. Clearance is also dependent on endothelial fenestral size. Fenestrae are highly variable, soit is difficult to determine the efficacy and toxicity of nanomedicines in different individuals because age, sex and genetics influence their rate of clearance. Whether nanocarriers are taken up by macrophages depends on opsonization by the innate immune system. Opsonins, molecules that bind to foreign materials and enhance phagocytosis, include IgG and IgA antibodies, the complement cascade system and mannose-binding lectin [1]. Therefore, the surface properties of nanocarriers can significantly affect the rate of clearance by the MPS. A useful method for evading opsonization of large narrow carriers was developed in Rutgers University in the 1960s in a process called PEGylation, a polymer, poly (ethylene glycol) (PEG; $[CH_2CH_2O]n$), is conjugated to the drug carrier [1]. Overall, use of ligand–drug–nanocarrier complexes improves the drug therapeutic index. The high selectivity and specificity of the complex increase the amount of drug delivered to the target tissue and decrease the amount at unwanted sites. Therefore, less systemic drug needs to be administered to ensure a sufficient concentration at the site of action and the minimum efficacious dose is also lower. In addition, because less drug is present at unwanted sites, the maximum nontoxic is higher. The overall effect is a drastic decrease in toxicity and adverse side effects.

The liposome bilayer

The liposome bilayer can be composed of either synthetic or natural phospholipids. The predominant physical and chemical properties of a liposome are based on the net properties of the constituent phospholipids, including permeability, charge density and steric hindrance. The lipid bilayer closes in on itself due to interactions between water molecules and the hydrophobic phosphate groups of the phospholipids. This process of liposome formation is spontaneous because the amphiphilic phospholipids self-associate into bilayers. Drug loading into liposomes can be achieved through liposome formation in an aqueous solution saturated with soluble drug; the use of organic solvents and solvent exchange mechanisms; the use of lipophilic drugs; and pH gradient methods [1]. 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. 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 reduce 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. Liposomal formulations of anticancer drugs have already been approved for human use. Doxilis a liposomal formulation of the anthracycline drug doxorubicin used to treat cancer in AIDS-related Kaposi sarcoma and multiple myeloma [2]. Its advantages over free doxorubicin are greater efficacy and lower cardiotoxicity. These advantages are attributed to passive targeting of tumors, due to leaky tumor vasculature and the EPR effect, and to lower concentrations of free doxorubicin at healthy tissue sites. There is evidence that liposomal Doxil1 is metabolized by leukaemia cells via a different mechanism than that for free doxorubicin, which might explain the improved efficacy and lower toxicity. Furthermore, Doxil is under clinical trial for the treatment of breast cancer [3]. One of the most interesting developments in this field is the potential of solid lipid NPs to combat the increasing problem of multidrug resistance (MDR) acquired by cancers, which drastically reduces chemotherapeutic efficacy. Proposed mechanisms underlying MDR at the cellular level include: increased metabolism of drugs due to increased enzyme expression, especially of glutathione S-transferase; drug transporters and efflux protein and point mutations in proteins that are therapeutic or drug targets. The following articles elucidate the previous research regarding these particles as well as what the future of this technology may hold.

Nano particulate drug delivery systems for cancer treatment

Initially, researchers saw the potential in drug delivery systems to enhance the therapeutic index of certain anticancer agents by either increasing the concentration of a given drug in tumor cells or decreasing the exposure in normal host tissues. Breast cancer, a solid tumor contains a variety of challenges to traditional therapies [4]. Drug penetration being a key issue as solid tumors generally have a heterogenous vascular supply and high interstitial pressures within tumor tissue. Drug delivery systems can often make these issues far worse from slow diffusion of therapeutic agents through the tissues. Some delivery systems have been developed to combat this via the enhanced permeability and retention effect. This effect refers to the result of a dysregulation of tumor angiogenesis, often leading to defects in tumor physiology resulting in increased permeability. Therapeutic agents with increasingly restricted volumes of distribution and the ability for prolonger circulation typically preferentially diffuse from these abnormal vessels and accumulate within the tissue of a tumor. Long circulating liposomal drugs rely on this mechanism of action in anticancer treatment (Park, 2002). Anthracyclines represent one particular avenue of anticancer therapy with liposomes. Most versions utilize ion trapping methods in order to provide effective loading of drugs

such as doxorubicin or daunorubicin within the aqueous layer of unilamellar liposomes [4]. Liposomal encapsulation of these drugs was found to significantly reduce toxic effects by altering the drug biodistribution.

Cytotoxic drugs used in cancer chemotherapy

Many cytotoxic drugs used in cancer chemotherapy have a very narrow therapeutic index, thus typically result in serious side effects, affecting optimal dosage calculations and reducing patient compliance. Even though drug research has made tremendous strides, cancer chemotherapy has largely remained nonspecific, thus most drugs available are toxic to normal and tumor cells alike. Several drug carriers have attempted to solve this through the use of molecular conjugates and colloidal particulates in order to modify the biodistribution of cytotoxic drugs, thus improving selectivity for tumors and reducing any damage to normal tissues [5]. Molecular conjugates are typically obtained by chemically linking a given active molecule to a targeted vector such as a complex carbohydrate, polymer, polypeptide, or antibody. Colloidal particulates are the result of physical incorporation of a drug of biologically active molecule into a specific particulate colloidal system. Liposomes are the most frequently studied particulate system as their composition holds great promise in the delivery of anticancer agents. Several advantages of these nanoparticles include their structural resemblance to cell membranes, leading to metabolic compatibility, low toxicity, and lack of immune response [5]. Also, their ability to encapsulate an aqueous compound within a lipid bilayer suggests an ability to deliver and encapsulate both hydrophilic and lipophilic drugs. The article meticulously explains the difference between the various types of liposomes such as conventional liposomes, long-circulating liposomes, sterically stabilized liposomes, and pegylated liposomal doxorubicin [5]. Liposomes have been demonstrated as a very successful nonviral vector agent for gene delivery as well, researchers being very interested on plasmid-liposome complexes and how they can be utilized as immunotherapeutic agents to treat metastatic melanoma, renal cell and colorectal cancer [5]. Cationic liposomes have a variety of advantages as gene transfer agents as well as viral-based vectors in the future.

Liposomes is pancreatic cancer

Another cancer area of interest when it comes to liposomes is pancreatic cancer. Notably one of the most difficult human malignancies to treat, the mortality rate is almost equal to its incidence. Adjuvant chemotherapy typically demonstrates improvements in disease free survival and overall survival rates thus making postoperative chemotherapy considered a standard of care for patients with pancreatic cancer. Gemcitabine is the established standard fist line of care treatment for advanced stage pancreatic cancer, however not much has been done to improve treatment outcomes in recent years. Pancreatic cancer cells possess an incredible amount of chemoresistance, thus impairing drug delivery and resulting in poor biological half-life of chemotherapeutic drugs [6]. Gemcitabine is thus considered far from optimal and many researchers support the urgent necessity of developing novel therapeutic strategies to combat these challenges. Developments in liposomal drug delivery systems have aided in increasing the specificity of therapeutic agents for pancreatic cancer. Novel tumor targeting liposomal complexes loaded with conventional MRI contrast agents and fluorescent dyes have resulted in increased resolution and image intensity in orthotopic mouse models of pancreatic cancer [6]. These systems could be further developed as different platforms for multifunctional theranostic nanodevices can be specifically made for early cancer detection as well as functional drug delivery. A new liposomal formulation of gemcitabine has been developed with far better pharmacodynamics and pharmacokinetics than conventional gemcitabine as well as increased antitumoral activity in orthotopic mouse models [6]. The anticancer potential of these gemcitabine loaded liposomes could result in prolonged circulation time and advantages in terms of intracellular drug entrance and accumulation within tumor tissues.

Efficacy of nano drug carriers

Drug carrier behavior influenced remotely has been considered a major enhancement to the efficacy of drug delivery to target sites. Liposomes being the ideal method of delivery with its high biocompatibility and biodegradability as well as the ability to deliver pharmaceutical agents into cells and individual compartments within tissues. Novel liposomes have been developed to respond to environmental stimuli within the body to release encapsulated contents [7]. This interaction has been well studied and documented based on the relationship between the stimuli and the liposome, with a magnetic triggered system becoming a potential strategy with great promise. Specifically iron oxide based magnetic nanoparticles are the primary choice due to the physical properties and biocompatible nature of the compound. These nanoparticles demonstrated hydrophilicity and stability within an aqueous environment, thus aqueous colloidal dispersions are the main applications of this technology [7]. This system provides the characteristics of both passive and active targeting, without modification of the nanoparticle surface as well as direct targeting to the given site of interest within the tissue. Magnetic nanoparticles typically respond strongly to magnetic fields and thus can penetrate human tissue without much issue. Once accumulated within the tissue, these nanoparticles can induce hyperthermia by using exothermic properties from hysteresis or Neel relaxation losses from a high frequency magnetic field to increase the temperature around surrounding cells [7]. Integrating this system with liposomes has yielded great promise in the delivery of doxorubicin in colorectal cancer patients. The study demonstrated the formulation exhibited no cytotoxicity against the cellular system, but would in fact synergistically increase the cytotoxic effects in order to kill colorectal cells [7]. Thus, the researchers postulated that the combination of chemotherapy and hyperthermia would be the ideal combination for cancer treatment in the future.

Liposomes continue to be studied and developed for anticancer treatment. One such study investigated the novel dual functional drug liposomes in which the basic efficacy of a drug is provided, and the effects are extended [8]. As an intensified iteration of targeting liposomes, the dual function occurs during the delivery and action process enhancing the potential applications in drug resistant cancers. Multidrug resistance of cancer has arisen as a major challenge as the resistance of cancer cells to one chemotherapeutic drug is followed by resistance to other drugs with different structures and action mechanisms altogether. Examples of such cancers include leukemia, breast cancer, lung cancer, and brain glioma among others [8]. Dual functioning drug liposomes were found to produce much better outcomes by incorporating anticancer drugs with a multidrug resistance inhibitor and block adenosine triphosphate binding cassette transporters during treatment as demonstrated using dual functioning liposomes by coencapsulating paclitaxel chloroquine to treat paclitaxel resistant carcinoma [8]. Researchers

then confirmed these nanoparticles were able to block the efflux of paclitaxel by adenosine triphosphate binding cassette transporters. Dual functioning drug liposomes were also found to enhance drug penetration across biological barriers where adenosine triphosphate binding cassette transporters were overexpressed such as in the blood brain barrier. In comparison to traditional drug liposomes, the dual functional drug liposomes extended the function of the liposomes as well as improved drug efficacy making it a promising potential in the treatment of drug resistant cancer. The biocompatibility, long circulation, and low systemic toxicity would enable a variety of applications in clinical treatment as well as their extended function.

FDA approved liposomal drug delivery systems

Currently there are several liposomal anticancer drugs as authorized by the United States Food and Drug Administration, such as doxorubicin. The liposomal formulation contains polyethylene glycol coated liposomal doxorubicin as an option in the treatment of AIDS-related Kaposi's sarcoma, metastatic breast cancer, advanced ovarian cancer, and relapsed or refractory multiple myelomas [9]. Cationic liposomes are typically used for transfecting cultured cells in which they are able to release their contents into the cell. Liposomal fusion is the mechanism heavily relied on as charged lipid bodies are neutralized as a result of pH changes or the presence of neutralizing multivalent ions. Charge neutralization on the surface of the lipids leads to dehydration of lipid bilayers leading to membrane fusion, resulting ultimately in non-bilayer lipid intermediates [9]. By better understanding the lipid properties, researchers are able to gain better insight into lipid mediated transfection that can be useful in explaining the variations within the transfection of different cell lines. If the drug is not targeted specifically to cancer cells, it could continue to have a significant contribution in the treatment, however the drug could cause unforeseen side effects and toxicity to noncancerous cells and tissues. To prevent this, researchers compared saturated and unsaturated lipids within stable doxorubicin loaded PEGylated liposomes in breast cancer [9]. The study was able to demonstrate the increased efficacy of doxorubicin loaded liposomes on breast cancer as well as reducing the dosage dependent side effects and enhance the tumor specific toxicity of doxorubicin.

Immune liposomes by combining immunotherapy and chemotherapy

Another study considered how triple negative breast cancer could be combated using detachable immune liposomes by combining immunotherapy and chemotherapy [10]. Their model was the mechanism of innate immune cell type tumor associated macrophages and thus developed a matrix metalloprotease responsive integrated immunochemotherapeutic strategy in order to deliver paclitaxel by detachable immune liposomes. aCD47 was conjugated to liposomes via a matrix metalloprotease 2 responsive phospholipid to induce immunotherapy while paclitaxel was loaded into phospholipid bilayers to trigger chemotherapy against the tumor tissue. Cytotoxicity experiments showed paclitaxel loaded liposomes with stronger inhibition compares to free paclitaxel as well as higher apoptosis rates [10]. These nanoparticles were able to significantly inhibit cell migration and proliferation through the immune efficacy in conjunction with the chemotherapeutic effect, showing great promise for antitumor efficacy *in vitro*. Overall, researchers were able to develop a complex synergistic delivery of aCD47 and paclitaxel for immunochemotherapy of triple negative breast cancer using liposomes. Paclitaxel loaded liposomes rapidly released aCD47 in the tumor microenvironment via matrix metalloprotease 2 activity, which in turn polarized M2 macrophages into M1 macrophages to increase phagocytosis of tumor cells [10]. These nanoparticles strongly inhibited tumor cell proliferation as well as reducing the recurrence by relying on a systemic antitumor immune response promoted by antigen presentation on T cells.

Peptide functionalized liposomes represents a relatively new and emerging type of liposome used in anticancer treatment. These nanoparticles are typically tagged with some type of imagine agent for specific and effective delivery of diagnostic agents to the given site. Peptide liposomes targeting specific receptors, irradiation-mediated diagnostic imaging with peptide-targeting liposomes, and peptide-conjugated theranostic liposomes can possess both therapeutic and diagnostic effects which can be increasingly effective for early cancer detection [11]. Peptide conjugation to the liposomal system is a key step as it provides conserved peptide targeting functionality from its attachment on the outer layer of the liposome. Most peptides have a targeting effect on the tumor site via structural and biological functionality. Peptide functionalized liposomes maintain the stability of the peptide drug as well as facilitate the targeted delivery of liposomal components into the desired tumor site. Different drug molecules are then encapsulated or attached along with the peptide functionalized liposomes for the synergistic effect in different treatment purposes, such as multidrug resistance [11]. Most of the liposomes that are clinically approved and have been manufactured on a large scale are very well characterized, which was possible due to their simple composition and structure. The scenario might not be the same in the large-scale production of these targeted liposomes. The functionalized liposomes have been studied mostly in a small laboratory setting where the product is a small amount. Shifting from the small scale to large scale comes with a massive challenge in the case of functionalized liposomes. The fact that it has been tremendously difficult to quantify the ligand conjugation with the liposomes accurately will undoubtedly lead to batch-to-batch variations in liposomes. This may result in variations in physicochemical properties of liposomes that affect their stability, biodistribution, and pharmacodynamics aspects of the functionalized liposomes [11].

Liposomes have the potential to enhance drug delivery to brain tumors and improve treatment effectiveness. They exert their therapeutic effect by releasing their cargo in specific regions of the tumor's vasculature and extravascular space [12]. Through targeted delivery, liposomes can be directed to specific areas of the blood brain barrier or glioblastoma multiforme tumors to deliver anti-cancer drugs. This section discusses several studies primarily aimed at translating these promising formulations into preclinical settings. In one study, the penetration of a commercially available glutathione DOX–PEGylated liposomal formulation through the blood brain barrier was investigated *in vitro* and *in vivo* using mice. The research demonstrated improved tumor regression in a murine model due to the increased delivery of doxorubicin from the targeted liposomes. In comparison to free doxorubicin (DOX), the pharmacokinetic profile showed that both 2B3-101 and PEGylated liposomal DOX were taken up at a higher rate *in vitro,* following incubation with 450 µg HSPC per mL of both liposomal formulations for 1 to 5. 5 h. Immediately after administration, PEG-lipo-DOX and 2B3-101 exhibited similar concentrations in blood plasma; however, there was a significant difference in plasma concentrations 21

h after treatment [12]. Biodistribution data revealed that DOX concentrations were highest in the brain following treatment with 2B3-101, approximately three times higher than in animals given free doxorubicin and 1.5 times higher than in animals given PEGylated liposomal DOX [12]. The median survival time for animals receiving saline treatment was 13 days. In contrast, animals receiving PEG-lipo-DOX had a median survival time of 15. 5 days. Animals receiving 2B3-101 had a median survival time of 18 days [12]. These results highlight the potential usefulness of targeted liposomal formulations, such as 2B3-101, in improving drug delivery to brain tumors and enhancing treatment efficacy. However, it is important to note that this study was conducted in preclinical models, and further research, including clinical trials, would be necessary to determine the safety and efficacy of these liposomal formulations in human patients. Nonetheless, these results demonstrate the potential usefulness of targeted liposomal formulations, such as 2B3-101, in improving drug delivery to brain tumors and enhancing treatment efficacy. They provide a promising foundation for future investigations and clinical trials aimed at developing effective liposomal-based therapies for brain cancer patients.

Another study was used to investigate the effects of focused ultrasound (FUS)-induced disruption of the BBB after the administration of liposomal doxorubicin (Lipo-DOX) *in vivo*. The study involved nine rats, five receiving Lipo-DOX combined with FUS, while the remaining four received FUS only. Subsequent examination of the brain tissue revealed a significant increase in the concentration of doxorubicin in the Lipo-DOX group compared to the FUS-only group [12]. These findings indicate that combining Lipo-DOX administration with FUS-induced BBB disruption leads to enhanced drug delivery to the brain tissue. The temporary disruption of the BBB caused by FUS allows the increased penetration of liposomal doxorubicin into the brain, resulting in higher drug concentrations at the target site.

In another study, researchers developed liposomes coated with d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), a PEGylated vitamin E, and loaded them with docetaxel (DTX) as a model drug for the improved treatment of brain tumors [12]. The liposomes were synthesized using the solvent injection method, and their mean particle size ranged from 126 to 191 nm. The drug encapsulation efficiency was 64. 1±0. 6% after 24 h of dialysis in PBS. After 24 h of cultivation with C6 glioma cells, the IC50 (drug concentration required to kill 50% of cells) was determined to be 7.04±1.05, 31.04±0.75, 7.70±0.22, and 5.93±0.57 μg/mL for Taxotere, naked liposomes, PEG-coated liposomes, and TPGS-coated liposomes, respectively [12]. The study concluded that TPGS-coated liposomes exhibited significant improvements *in vitro* compared to PEG-coated liposomes. These studies contribute to understanding liposomal drug delivery systems in the context of brain tumor treatment. Researchers demonstrated the potential of combining liposomal DOX with FUS-induced blood brain barrier disruption to enhance drug concentration in the brain, while Muthu et al. research highlighted the advantages of TPGS-coated liposomes in terms of drug encapsulation efficiency and improved efficacy *in vitro*. However, further studies, including clinical trials, would be necessary to assess the safety and efficacy of these approaches in human patients.

In a similar investigation, theranostic liposomes decorated with RGD-TPGS and co-loaded with docetaxel and QDs were tested *in vivo* on rats. These liposomes had a mean particle size of 175.6±3.2 nm. The release of the drug from the liposomes after 72 h of dialysis in PBS at pH 7.4 was 68.41±3.56% [12]. After 2 h and 4 h of therapy, the RGD-TPGS-decorated theranostic liposomes exhibited 6.47-fold and 6.98-fold higher efficacy than Docel *in vivo*. The liposomes were internalized into tumor cells through RME, where the liposomes were synthesized by solvent injection method. Moreover, the RGD-TPGS-decorated theranostic liposomes effectively reduced ROS production and showed no evidence of brain injury or edema in brain histopathology. Targeted liposomes, such as those decorated with RGD-TPGS, show promise in enhancing the efficacy and specificity of drug delivery to brain tumor cells. Future research should focus on developing even more precise targeting strategies by exploring other ligands or receptor-specific molecules that can further improve the selectivity of liposomes toward tumor cells.

In another study, thermosensitive magnetic liposomes (TML) encapsulating Camptosar (CPT-11), coated with magnetic Fe_3O_4 nanoparticles, and conjugated with Cetuximab (CET) were synthesized. These liposomes demonstrated high biocompatibility and enhanced intracellular uptake by human primary glioblastoma cells (U87) when exposed to a high frequency alternating magnetic field (AMF). *In vivo* studies demonstrated the efficacy of these liposomes, where the tumor sizes reported for the experimental group on days 14,17, and 21 after liposome administration were 1.9 mm^3, 27.1 mm^3, and 76.3 mm^3, respectively, compared to the control group with tumor sizes of 29.1 mm^3, 105.9 mm^3, and 109.5 mm^3, respectively [12]. The liposomes were internalized into the cells using the RME pathway and were synthesized using the freeze–thaw method. These studies highlight the potential of using targeted and theranostic liposomes for drug delivery in brain tumor treatment. The RGD-TPGS-decorated liposomes demonstrated enhanced efficacy and specificity, with the ability to reduce ROS production and minimize brain injury. Similarly, the thermosensitive magnetic liposomes exhibited improved intracellular uptake and significant tumor size reduction *in vivo*. These findings contribute to the development of novel strategies for targeted drug delivery and theranostics in brain tumor therapy. Likewise, the co-loading of drugs within liposomes, as demonstrated in the studies, presents opportunities for combination therapies. Combinations of chemotherapeutic agents, targeted therapies, immunotherapies, or other treatment modalities can be encapsulated within liposomes to create synergistic effects and enhance therapeutic outcomes. While the studies mentioned provide promising results in preclinical models, further research is necessary to validate the safety, efficacy, and scalability of these liposomal formulations for clinical use. Future perspectives may involve conducting clinical trials to evaluate the performance and therapeutic benefits of targeted and theranostic liposomes in patients with brain tumors, aiming to improve treatment outcomes and patient quality of life. Moreover, further research may focus on identifying optimal drug combinations and exploring the potential synergies that can be achieved with theranostic liposomes.

Another group developed TMZ-loaded liposomes and utilized ultrasound-mediated BBB permeabilizing technology to achieve localized delivery of these nanoparticles into GBM. In an *in vitro* BBB model, TMZ-liposomes demonstrated significantly higher efficacy in killing C6 tumor cells when combined with ultrasound (US) irradiation compared to free TMZ alone [12]. In mice treated with US-mediated blood brain barrier opening, TMZ-liposomes were transcytosed more efficiently due to FUS-induced BBB disruption. Focused ultrasound penetrates the skull and generates microbubbles in the blood, leading to

various cavitation effects near the BBB and within the GBM. This resulted in substantial suppression of tumor growth and prolonged animal survival. The liposomes were internalized into the cells using the RMT pathway and were synthesized using the thin-film hydration method. The localized delivery achieved with the application of TMZ-loaded liposomes with US irradiation showed superior tumor growth suppression and increased animal survival. The combination of TMZ-loaded liposomes with ultrasound-mediated BBB permeabilization demonstrated enhanced localized drug delivery to GBM tumors. This approach uses focused ultrasound to temporarily disrupt the BBB, allowing liposomes to cross into the brain. Future research may focus on optimizing the ultrasound parameters and developing strategies to improve the efficiency and safety of this technique for clinical applications.

In another study, DOX-containing liposomes conjugated with both transferrin (Tf) and folate were developed to enhance the transport of DOX across the BBB and into brain gliomas. The liposomes had an average particle size of 180 nm ± 12.5 nm [12]. DOX was efficiently entrapped in the Tf-DOX-liposomes with an encapsulation efficiency of 94.5% ± 0.5%. The liposomes were able to cross the BBB through receptor-mediated endocytosis. The dual-targeting strategy significantly improved the survival of mice with brain tumors and reduced cytotoxicity compared to the groups treated with the free drug. Similarly, the dual-targeting approach employing DOX-containing liposomes conjugated with transferrin (Tf) and folate has shown enhanced transport across the BBB and improved therapeutic outcomes in models of brain glioma. This dual-targeting strategy exploits the overexpression of Tf and folate receptors on tumor cells to enhance liposome uptake. Further investigations may explore the potential of other targeting ligands or receptor-specific molecules that can improve the efficiency of liposomal transport across the BBB and increase tumor accumulation. The studies mentioned provide promising results in preclinical models, highlighting the potential of liposomal drug delivery for brain tumor therapy. Future perspectives may involve conducting rigorous preclinical studies to further evaluate the safety and efficacy of liposomal formulations. Subsequently, clinical trials should be conducted to assess the performance of liposomes in patients with brain tumors and to determine their impact on patient outcomes.

A different study investigated the anti-cancer efficacy of PEGylated liposome nanoparticles (PEG-Lip) loaded with the chemotherapy drugs doxorubicin and carboplatin (CB) for treating brain cancer. The synthesized liposomes had a size of 212 nm ± 10 nm. *In vitro* release studies at pH 7.4 demonstrated controlled release of the encapsulated drugs, with PEG-Lip-DOX, PEG-Lip-CB, and PEG-Lip-DOX/CB releasing 79.3%, 76.7%, and 69.3% of the loaded drugs, respectively, in 52 h [12]. PEG-Lip-DOX/CB exhibited 1.5-fold higher cytotoxicity and 1.3-fold higher reactive oxygen species (ROS) generation compared to Lip-DOX/CB. In glioblastoma-bearing rats, PEG-Lip-DOX/CB significantly increased survival time by 23.1% and 10.2% compared to DOX + CB and Lip-DOX/CB, respectively. The liposomes were internalized into the cells using the RME pathway and were synthesized using the thin-film hydration method. The improved cytotoxicity and increased reactive oxygen species (ROS) generation observed with PEG-Lip-DOX/CB suggest a potential synergistic effect of the drug combination within the liposomal formulation. Moreover, the significant increase in survival time in glioblastoma-bearing rats treated with PEG-Lip-DOX/CB highlights the therapeutic potential of this liposomal system.

In another study, liposomes coupled with various cell-penetrating peptides (CPPs) were investigated as a gene vector delivery system across the BBB. Liposomes conjugated with CPPs and transferrin (Tf) ligands were synthesized, and their impact on liposome transport capacity and transfection effectiveness in brain endothelial cells was studied. The liposomes had a size of 155 nm and an encapsulation efficiency of 84.6%. *In vitro* studies demonstrated increased uptake in brain endothelial cells, with uptake levels reaching 33% and 71% after 1 h and 4 h of incubation, respectively [12]. *In vivo* biodistribution studies in mice showed that 7.7% of the administered dosage was able to penetrate the BBB and accumulate in brain tumors via endocytosis. The increased uptake in brain endothelial cells and the accumulation of liposomes in brain tumors demonstrated in *in vitro* and *in vivo* experiments indicate the potential of these liposomes as gene delivery vectors for brain cancer treatment. These studies emphasize the versatility and potential of liposomal drug delivery systems for brain cancer therapy. The ability to optimize liposome characteristics, incorporate targeting ligands, and achieve controlled release of drugs provides opportunities to overcome the challenges associated with brain tumor treatment. Further research and development in this field hold promise to translate these findings into clinical applications, offering improved treatment options for patients with brain cancer.

Conclusion

Liposomes constitute the majority of clinically approved nanocarriers for anticancer agents. Liposomes have been esteemed for their favorable attributes; they provide a wealth of opportunities for their extensive therapeutic pharmaceutical applications as drug delivery systems, particularly in the treatment and diagnosis of cancer. Long-circulating liposomes are currently gaining recognition and clinical approval for their ability to improve drug delivery to target tissues. Liposomes have been acknowledged for their ability to selectively target diseased tissues through functionalization with targeting moieties. Furthermore, the appeal of liposomes lies in their biocompatibility and reduced drug clearance. Although liposomes are generally safe and intrinsically low in toxicity, efforts should be made to examine the environmental and toxicological impact of the long-term exposure of liposomes in humans and animals. Nevertheless, liposomes have proven to be promising drug delivery systems as evidenced by the widespread success of currently marketed liposomal products.

In order to determine the best avenue for further research, understanding the different types of liposomes and their effects is crucial. Anticancer therapy relies on targeted drug delivery systems and liposomes have demonstrated great promise within this field, however much of the current research focuses heavily on the different targeting groups that can be inserted into the phospholipid layer. It would be best to consider the innate structures at play within these targeting groups and how they can become better suited and positioned to improve the efficacy of liposomal drug delivery. With the various types of cancers analyzed and discussed, the potential of liposomes within the field of nanotechnology is incredible, with many questions left unanswered. How can liposomes become better adapted to specific targeted drug delivery systems? What are the best drugs that have been implemented in the past using this technology and how will pharmaceutical agents become better adapted into these systems in the future? A large part in solving these questions and others that will undoubtedly

arise are first and foremost developing a sound system through which researchers can understand the current options when it comes to liposomal drug delivery and how patients have been affected so far.

Future trends: The liposomes present significant potential for applications in the targeted delivery of chemotherapeutics in the treatment of cancer. Based on their potential, several formulations are already approved and are clinically used in cancer treatment. Targeting tumors with liposome nanocarriers could not only increase the therapeutic benefit and minimize associated toxicity but could also enhance the curative effect by specifically releasing the therapeutic agent. Chemotherapeutic drugs by themselves cannot differentiate the tumor cells and healthy cells. This leads to unwanted damage to the healthy cells and unfavorable bio-distribution of a drug in the body resulting in a suboptimal performance at the desired site of action. In addition, resistance to drugs by cancerous cells due to drug efflux transporters remains to be another major obstacle in the treatment of cancer. Liposomes gained attention as drug carriers in cancer therapy being nonimmunogenic, biocompatible, able to enhance drug solubility, and able to encapsulate a broad range of drugs. Passively targeted liposomes were successful in being translated to clinical use which can be substantiated by commercially available liposomal therapies. However, the translation of active targeted liposomes to patients either has faced shortcomings or is still in the clinical trials and needs to prove effective in clinical settings. By better understanding the options, efficacy, bioavailability, and possible side effects, researchers could aid clinicians in providing safe and effective anticancer therapy targeted specifically for real world drug delivery systems. The implications of the research would largely be providing a better method of determining which drug delivery systems work best with the current pharmaceutical agents and what mechanisms must be altered within the process. With increasing obstacles such as multidrug resistance and cancer mutations, researchers and clinicians alike must work together to provide solutions and better remedies and therapies for cancer patients. In the future, chemotherapeutic and immunotherapeutic agents must be delivered in a timely and targeted manner and liposomes could become the next useful tool in understanding and improving the approach to anticancer therapy. Determining the best approaches within the current system is key to mitigating issues within the current system and developing a better set of methods in the future.

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