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Review Article

Nanocarrier-Based Drug Delivery in Cholangiocarcinoma: The Role of Liposomes

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ABSTRACT

Cholangiocarcinoma (CCA) is an uncommon but extremely aggressive cancer that starts in the bile ducts' epithelial cells. CCA has a poor prognosis and few treatment choices because of its asymptomatic early stages, delayed diagnosis, and resistance to traditional chemotherapy. A promising tactic to increase the effectiveness and safety of chemotherapeutic drugs in the treatment of CCA, liposome-based drug delivery systems have attracted more and more interest in recent years. In this study, the enhanced permeability and retention (EPR) impact of liposomes is discussed in relation to the treatment of cholangiocarcinoma, emphasizing the benefits of targeted drug delivery and tumor-specific accumulation. We investigate several liposomal formulations, such as those functionalized with antibodies or ligands to actively target CCA cells. Tumor heterogeneity, the immunosuppressive tumor microenvironment, and the requirement for more thorough clinical validation are some of the obstacles that still exist in spite of these developments. To fully realize their potential in improving outcomes for patients with cholangiocarcinoma, this study highlights the need for ongoing research and innovation in liposomal technologies as well as customized medicine methods.

INTRODUCTION

Cholangiocarcinomas are malignancies of the biliary duct system that may originate in the liver and extrahepatic bile ducts, which terminate at the ampulla of Vater (1). This is the second most prevalent type of hepatic cancer after hepatocellular carcinoma (HCC), and it is a rare, extremely deadly epithelial cell malignancy that can develop anywhere along the biliary system and/or within the hepatic parenchyma (2). Shows the features of cholangiocyte formation as it develops from bile duct epithelial cells and their progenitor cells, resulting in a collection of

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dynamic, varied and heterogeneous cells lining the biliary tree (3). The word "CCA" refers to bile duct cancers that are either intra- or extra-hepatic (perihilar (pCCA) vs. distal (dCCA) (4). The classification of these malignancies based on anatomical location comprises intrahepatic, perihilar, and distal cholangiocarcinoma (5). Intrahepatic restricts to the common bile duct below the cystic duct insertion, perihilar is limited between the second-order bile ducts and the insertion of the cystic duct into the common bile duct, and distal is positioned proximally to the second-order bile ducts within the liver parenchyma (6). 10%–20% of cases were intrahepatic, 50% were perihilar, and 30%–40% were distal.The 60s and 70s are the most common age groups for both males and females with cholangiocarcinoma. Cholangiocarcinoma has a male-to-female ratio of 1:2.5 among patients in their 60s and 70s and 1:15 in patients under the age of 40(7). Adenocarcinomas make up the majority of CCAs, however adenosquamous carcinomas and clear cell carcinomas are infrequent. The heavy networks of inflammatory cells and matrix that make up a tumor's immune microenvironment become connected with these malignancies, which are extremely desmoplastic (8). Adenocarcinomas make up the majority of CCAs, however adenosquamous carcinomas and clear cell carcinomas are infrequent. The heavy networks of inflammatory cells and matrix that make up a tumor's immune microenvironment become connected with these malignancies, which are extremely desmoplastic (9). Due to its aggressiveness, late recognition, and immunoregulatory capacity, this tumor still has a high mortality rate; it is often ever detected at an early stage if nonspecific signs, such as painless jaundice, weight loss, or cholangitis, appear. Because these malignancies are challenging to identify and treat, the prognosis is unpredictable; most untreated patients might die within 3–4 months as a result of local tumor development, bile duct obstruction, liver failure, or sepsis from cholangitis and abscesses. The incidence of intrahepatic CCA, however, has risen substantially over the previous 10 years by 109%, from 0.67 per 100 000 in 2007 to 1.40 per 100 000 in 2016. Over the last few decades, the rate has risen everywhere. Without considering into account the Asian regions with the most frequency (> 6 per 100,000 individuals annually), shows the results for 2020 fall within 1-6 per 100,000 people annually. More than 95% of these tumors are ductal adenocarcinomas so we have to focus on patients present with unresectable or metastatic disease (10).

EPIDEMIOLOGY:

The prevalence of CCA is intimately correlated with changes in both the natural and constructed environments. In some areas, particular trematode infections—flatworm parasites commonly referred to as flukes—are significant contributors to CCA (11). In this case, the liver fluke Opisthorchis viverrini is the main contributor to CCA in Southeast Asia (12). Although CCA caused by a fluke may have a unique etiology, such as genetic variations, the diagnosis and treatment are similar as for CCA not caused by a fluke (13).

- **Fluke-related CCA**

One of the main causes of CCA in certain geographic regions is an infection with a particular trematode. These flatworm parasites are group 1 biological carcinogens, which means they have been reported to cause cancer (14). This category includes many fish-borne liver flukes which belong to the Opisthorchiidae family. Food-borne trematodes O. Viverrini and the related liver fluke C. Sinensis have life cycles which are similar and begin when humans and other fish-eating mammals, the definitive hosts, release eggs into

the environment through their feces. Freshwater snails (Bithynia spp. or Parafossarulus spp.) act as the miracidia's first intermediate host when their eggs reach freshwater, where they hatch and undergo transformation as sporocysts, rediae, and cercariae. The second intermediate host is freshwater fish (Cyclocheilichthys spp., Puntius spp., and Hampala dispar). When humans ingest raw or undercooked cyprinoid fish products, the cercariae that encyst as metacercariae in the muscles or under the scales may infect them (15). The metacercariae move by the ampulla of Vater, reach the human small intestine unharmed and settle in the bile ducts, where they finally develop into adult worms within 4 weeks and lay yellow, operculated eggs (16).

- **Non-fluke-related CCA**

Multiple investigations have found an ongoing pattern of rising (iCCA) rates and stable or decreased (pCCA) and/or (dCCA) rates (17). Cholangiocytes activation, apoptosis, senescence pathway progression, and enhanced cellular turnover are caused by pancreatic enzyme reflux, cholestasis, and chronic inflammation. The progression of cancer involves each of these mechanisms: A shared pathway (including interleukin 6, cyclooxygenase-2, nitric oxide, etc.) between inflammation and malignant cellular proliferation that affects hepatic progenitor cells has been highlighted by certain research (18). An alternative carcinogenetic theory has been presented along this pathogenetic idea, It is based on the activation of the mitogenic pathway, leading to the multistep development of the tumor (19).

ETIOLOGY:

Cancer arises when there is a build-up of mutations in essential genes, including those that manage cell division, which causes cells to expand and divide uncontrollably to create a tumor (20). These genetic alterations (somatic mutations) are acquired all throughout the course of a person's lifetime and are solely present in the bile duct cells that give rise to the tumor in the majority of cases of cholangiocarcinoma (21). Genes serve as suppressors of tumors, which means they promote tightly regulated cell growth and division. Tumor suppressor gene mutations or deletions can cause excessive or disorderly cell growth and division, which is a characteristic of cancer (22). When cells in the bile ducts have DNA modification's, cholangiocarcinoma result. The modifications direct the cells to proliferate excessively and aggregate into a mass of cells (tumor) that may penetrate and disrupt healthy human tissue (23).

PATHOPHYSIOLOGY:

Apparently the extrahepatic or intrahepatic biliary epithelium may rise to cholangiocarcinomas. The remainder are squamous cell cancers, of which over 90 percent are adenocarcinomas (24). A large number of bile duct tumors have unknown causes. Chronic inflammation has been proposed to play a role by promoting hyperplasia, cellular proliferation, and, ultimately, malignant transformation, such as in primary sclerosing cholangitis (PSC) or chronic parasite infection (25).

Chronic cholecystitis and chronic ulcerative colitis may be associated with intrahepatic cholangiocarcinoma.Cholangiocarcinomas typically grow slowly, go through the duct walls, and spread along tissue planes (26). Local development of the celiac and pancreaticoduodenal chains occurs into the liver, porta hepatis, and local lymph nodes (16). Cholangitis, an occasionally fatal illness, may develop and call for early antibiotic therapy and aggressive biliary drainage (27).

MOLECULAR PATHOGENESIS:

A wide range of risk factors result in persistent inflammation or cholestasis, regardless of the etiology. Cholangiocarcinomas often develop slowly, penetrate the duct walls, and divide along tissue planes. Local development of the celiac and pancreaticoduodenal chains occurs into the liver, porta hepatis, and local lymph nodes. The inflammatory mediator's interleukin-6, necrosis factor-, cyclo-oxygenase-2, and Wnt were exposed to cholangiocytes progressively as a result of chronic inflammation, triggering increasing modifications in DNA mismatch repair, tumor suppressor, and proto-oncogene genes (28). Persistence of inflammation has been claimed to play a role by promoting hyperplasia, cellular proliferation, and, ultimately, malignant transformation, as in primary sclerosing cholangitis (PSC) or constant parasite infection. Chronic cholecystitis and chronic ulcerative colitis may be associated with intrahepatic cholangiocarcinoma. A buildup of bile acids in cholestasis causes a reduction in pH, a rise in apoptosis, and activation of the ERK1/2, Akt, and NF-b pathways, that enhance cell growth, migration, and survival. Transforming Growth Factor- Vascular Endothelial Growth Factor, and Hepatocyte Growth Factor belong to the mediators that are increased in cholangiocarcinoma. Cholangitis, a potentially fatal illness, may develop and call for rapid antibiotic therapy and severe biliary drainage (29). Tumor growth, angiogenesis, and cell migration are stimulated by increased expression of the cell surface receptor c-Met, the glucose transporter GLUT-1, and the sodium iodide symporter. Cholangiocarcinogenesis is probably brought on by variations in the bile duct microenvironment as a result of these processes at the molecular level. New genetic variations in CCA have been discovered recently as a result of advancements in genomic profiling technology, giving an understanding of the underlying genetic mechanisms of cholangiocarcinogenesis (30).

SIGNS AND SYMPTOMS:

Initial symptoms of bile duct cancer often fail to show themselves. As the disease gets worse, cancerous cells first go to the liver's outermost layer before going on to organs close by, namely the duodenum, common bile duct, colon, stomach, diaphragm, etc. (31)

• Itching, which may happen when excessive bilirubin penetrates the skin;

• Fatigue;

• Unintentionally weight loss

• High temperature (fever) of 38C (100.4F) or above

• chills and shivering • continuous fatigue and feeling sick

• stomach (abdominal) discomfort and swelling - some patients feel a dull aching in the upper right side of their gut (32).

DIAGNOSIS:

Blood tests that evaluate the function of your liver can assist your doctor know more about the cause of the symptoms and signs. The total amount of bilirubin and alkaline phosphatase that the liver has released into the blood can be determined during this process by analyzing a sample of blood. These substances may be more prevalent than usual as a sign of liver illness, which is caused by bile duct cancer. Utilizing blood tests, your liver function will be assessed, and tumor-related symptoms will be searched (33). Tests consist of:

✓ Tests for liver function

- ✓ Testing of the blood for underlying gastrointestinal cancers, CEA and CA19-9(Tumor marker test)
- ✓ Alpha-Fetoprotein (AFP), a blood test used to spot potential cancer. It can be used to identify bile duct cancer along with liver cancer.

Imaging tests can help your doctor see your internal organs and look for signs of cholangiocarcinoma. Techniques used to diagnose bile duct cancer include:

1. **Ultrasound:**

The initial test for biliary blockage or suspected liver disease is typically ultrasound. Ultrasound, a high-energy sound wave, is utilized in this process to generate echoes by bouncing off internal organs or tissues, including the abdomen. As mass lesions, intrahepatic cholangiocarcinomas can be identified.The ultrasound will usually indicate, however, that the tiny bile ducts have enlarged. This is referred to as a "dilation of ducts."(34)

2. **Computerized Tomography (CT) scans:**

Computed tomography (CT) can show bile duct dilatation and a tumor mass, bile duct wall thickening, or intraductal tissue in exophytic, infiltrative, and polypoid cholangiocarcinomas, respectively. This procedure uses a computer linked to an x-ray machine to make a series of detailed pictures of areas inside the body, such as the abdomen, taken from different angles. A dye may be injected into a vein or swallowed to help the organs or tissues show up more clearly (35).

3. **Magnetic Resonance Imaging (MRI) combined with Magnetic Resonance Cholangiopancreatography (MRCP):**

This procedure uses a magnet, radio waves, and a computer to make a series of detailed pictures of areas inside the body. Due to its high contrast resolution, multiplanar capabilities, and ability to identify the parenchymal, biliary, and vascular extension, this is typically regarded as the modality of choice in the diagnosis of cholangiocarcinoma. In contrast to this procedure, which uses a magnet, radio waves, and a computer to create a series of detailed images of various body parts, MRCP creates images of the pancreas, liver, bile ducts, gallbladder, and pancreatic duct. This dye can be injected intravenously or delivered as a tablet or liquid for ingestion. When detecting biliary blockage, MRI offers > 90% sensitivity and specificity. To understand the differences between benign and malignant bile duct strictures, conventional unenhanced and contrast-enhanced MRI must be conducted in addition to MRCP 40 (36).

TREATMENT AND MANAGEMENT:

Only surgery can cure CC patients; unfortunately, only around one-third of patients have resectable disease upon diagnosis. This comprises the Whipple technique, partial hepatectomy, and bile duct removal. The following additional treatment possibilities for cholangiocarcinoma include:

- Stenting
- Photodynamic therapy (PDT)
- Pharmacotherapy
- **Stenting:**

To treat biliary blockage, stents can be implanted through percutaneous transhepatic cholangiography (PTC) or endoscopic retrograde cholangiopancreatography (ERCP). Stenting could minimize irritation and enhance quality of life (7). Malignant biliary obstruction is frequently treated with biliary stenting as a palliative treatment. In recent decades, major advances in

endoscopic or percutaneous stenting for unresectable CC have been made. Stents are usually used when a tumor cannot be removed surgically or when a patient isn't a candidate for surgery (37).

Stents can be made of plastic or metal. Usually, plastic stents occlude after three months and need to be replaced. While more expensive, metal stents extend to a bigger diameter and frequently maintain their patency for longer (38).

- **Photodynamic therapy:**

A photosensitizing substance is used in this therapy and is given before to the photoradiation process. Neoplastic tissue preferentially maintains photofrin due to its selective character. Laser application starts the activation process by converting the medication from the neutral ground state to its excited state at a particular wavelength of light. If oxygen is present, cytotoxic radical species immediately trigger apoptosis and tumor necrosis, killing dysplastic cells (39). Local vascular pathways are additionally impacted. In a mouse model, PDT was shown to reduce the amount of xenografted human cholangiocarcinoma tumours by 60%. The most anticipated potential complication is skin phototoxicity, yet there is also a significant possibility of serious bacterial cholangitis and liver infections that require post-interventional antibiotics (40). PDT has been shown to significantly enhance quality of life following PDT and stenting and to have a significant survival benefit in patients with unresectable cholangiocarcinoma (41).

- **Pharmacotherapy:**

A. Chemotherapy: - Drugs are used in (chemo) to kill cancer. Your tumor can be decreased via systemic chemotherapy, which is administered throughout your body, rendering surgery possible. Chemotherapy is used to improve the quality of life and alleviate cancer symptoms if the malignancy is too advanced for surgery (42). Techniques for administering chemotherapy directly to your bile duct**.** Transarterial chemoembolization (TACE), which inserts minuscule chemo beads into the blood arteries feeding the tumor via a catheter. To stop blood from reaching the tumor, the beads block the vessel. The beads also release chemo drugs at the same time to lessen the tumor size (43).

There are two main types of chemotherapy used to treat bile duct cancer.

1. Chemotherapy that is administered intravenously or intramuscularly penetrates the bloodstream and can reach cancer cells throughout the body is referred to as systemic chemotherapy. Management for bile duct cancer that is irreversible, metastatic, or recurring includes systemic chemotherapy. Gemcitabine and cisplatin, capecitabine and oxaliplatin (XELOX), gemcitabine and oxaliplatin (GEMOX), and gemcitabine and capecitabine are all possible chemotherapeutic combination (44).

2. Regional chemotherapy: When chemotherapy is injected directly into an organ or cavities of the body, such as the abdomen, the chemicals primarily strike the cancer cells present. Your oncologist may use chemotherapy, which is a form of treatment with medications that slow or stop the growth of rapidly dividing cells, to delay or stop the growth of cancer. This can apply for both healthy and rapidly multiplying cancer cells (45).

3. Targeted Therapy: -Targeted medication therapies focus on particular defects that are

common within cancer cells. Targeted medication therapies can kill cancer cells by avoiding these deviations. Your doctor may do a test on your cancer cells to see if or not targeted therapy can treat your cholangiocarcinoma (46). As a second-line therapy for cholangiocarcinoma with certain driver mutations, targeted medicines are now available. These include isocitrate dehydrogenase 1 (IDH1) inhibitors for IDH1-mutated cholangiocarcinoma and fibroblast growth factor receptor 2 (FGFR2)-selective tyrosine kinase inhibitors for advanced cholangiocarcinoma that included a FGFR2 gene fusion or rearrangement (47).

NOVEL DRUG THERAPY (LIPOSOMES).

Cancer is the second leading cause of death and constitutes a major public health burden worldwide (48). Several liposome-based formulations received approval by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), with many others in clinical trials. Liposomes have several advantages, including improved pharmacokinetic properties of the encapsulated drug, reduced systemic toxicity, extended circulation time, and targeted disposition in tumor sites due to the enhanced permeability and retention (EPR) mechanism (49).

The effectiveness of many chemotherapeutic drugs can be limited by their rapid metabolism, their toxic side effects, and the development of resistance (50). To overcome these limitations, nanoparticles, such as liposomes, have been used to improve the therapeutic efficacy of various chemotherapeutic drugs. Liposomes provide several advantages, including improved pharmacokinetic properties of the encapsulated drug, long circulation time, and passive targeting and disposition in tumors and inflammatory sites due to the enhanced permeability and retention (EPR) mechanism (51). They can also reduce systemic toxicity associated with the free drug. In addition, liposomes can improve the solubility of drugs and provide slow and sustained release of encapsulated drugs (52). However, it is worthy to note that despite their efficacy in treating various cancers, nanoparticles, including liposomes, still have some potential toxicity and lack specific targeting and disposition (53). Liposomes are considered to be one of the most successful drug-carrier systems, and several liposomal formulations are actively marketed or are in clinical trials (54). Liposomes result from the self-assembly of phospholipids in an aqueous media, resulting in closed bilayered structures with an aqueous cavity and one or more bilayer phospholipid membranes (55).

Phospholipids are the main components of cell membranes, which make them biocompatible. In addition, their amphiphilic properties enable self-assembly into bilayer membranes in aqueous environments (56). These unique properties make phospholipids suitable for drug delivery systems such as liposomes. Phospholipids are characterized by their phase transition temperature (T_C), which is the temperature at which phospholipids transit from gel crystalline to liquid crystalline states (57). The T_C depends on many factors, such as the nature of the polar head group of phospholipids, the length of their aliphatic chains, and the presence of unsaturation in their hydrocarbon chains (58). Liposomal formulations usually include cholesterol incorporated into the lipid bilayer to decrease membrane fluidity and control the rate of drug release. Cholesterol can reduce the rotational freedom of the phospholipid hydrocarbon chains, which limits liposome interactions with plasma proteins and subsequent loss of the encapsulated material (59). Cholesterol also plays an essential function in regulating the biophysical states of the phospholipids in the

liposomes by controlling the lipid organization and phase behavior. Cholesterol decreases the order of phospholipids in the crystalline gel phase and increases the order in the liquid crystalline phase (60). Studies have shown that adding cholesterol to liposomal formulations shifts the T_C of phospholipids to a lower temperature, and a cholesterol composition above 30% abolishes the T_C. Moreover, adding cholesterol increases the stability of liposomes and limits their leakage after systemic administration (61). The unique structure of liposomes allows hydrophilic drugs to be retained in the aqueous interior. Hydrophobic drugs are usually inserted into the liposome bilayer; however, caution should be taken with this approach because high drug concentrations can disrupt liposomes. Amphipathic drugs can also be encapsulated in liposomes, provided the drug is partitioned between bilayer and aqueous phases (62). Additional advantages of liposomes include biocompatibility, biodegradability, and decreased drug side effects (63). Liposomes allow for controlled drug release and protection from rapid metabolism and clearance. Liposomes are also associated with improved patient compliance because of a decreased frequency of drug administration as compared to unencapsulated drugs (64). Liposomes, like other nanoparticles, have some disadvantages, including possible carrier toxicity (65). Typically, the toxicity of liposomes is lower, as compared to other nanoparticles. Liposomes are primarily composed of phospholipids, and other lipids, that are generally recognized as safe (GRAS), as well as biocompatible, biodegradable, and non-immunogenic (66). Another limitation of liposomes is their preparation on a large industrial scale with reproducible properties (67). The stability of liposomes constitutes another limitation, and lyophilizing the produced lipid vesicles is one of the proposed solutions to overcome this limitation (68).

❖ Preparation and Properties of Liposomes

Most techniques used in liposome preparation include the dissolution of phospholipids in their appropriate organic solvents, followed by the removal of organic solvents to allow liposomes to form (69). There are several different methods used to load drugs into liposomes, and these differ depending on the hydrophilicity or hydrophobicity of the drug being encapsulated, as well as whether liposomes are manufactured at a small laboratory scale or an industrial scale (70). Hydrophobic drugs, such as paclitaxel and docetaxel, have been loaded into liposomes using the lipid film hydration method with sufficient encapsulation (71). Hydrophilic drugs are encapsulated into liposomes using passive or remote (active) loading. Passive loading involves entrapping drugs as the lipid films are hydrated; however, a major limitation of this method is the low encapsulation efficiency, as most hydrophilic drugs remain entrapped in the external aqueous compartment (72).

- **Thin Lipid Film Hydration.**

Thin lipid film hydration is the simplest, oldest, and one of the most widely used methods at the research laboratory scale (73). Phospholipids dissolved in organic solvents are subject to the removal of organic solvent via evaporation, resulting in a thin lipid film. Hydration of the lipid film results in heterogeneous liposomes dispersed in the aqueous solvent. Several techniques can reduce their heterogeneity and narrow their size distribution, including sonication and multiple extrusions through polycarbonate membranes (74).

- **Reverse Phase Evaporation.**

Reverse phase evaporation is a relatively simple method that is used to improve the EE of drugs into

liposomes (75). This method is based on the formation of an emulsion of an aqueous phase (containing the drug) and an organic phase (containing the lipid), followed by the evaporation of the organic solvent, and the formation of an aqueous suspension containing the assembled liposomes (76)

❖ Strategies for Targeting Liposomes to Tumors

Liposomes have emerged as efficient carrier systems for therapeutic agents, owing, in part, to some of the unique properties discussed above. Various strategies have been developed to target liposomes to tumor sites. Some of these strategies involve using passive targeting and active targeting via surface functionalization as well as using various stimuli to trigger drug release from liposomes.

Liposomes and the EPR Effect (Passive Targeting)

Nanoparticles, such as long circulating liposomes, take advantage of the leaky nature of the blood vessels in tumor tissues. Because tumor blood vessels have inceased fenestrations, liposomes can passively cross the capillary endothelial barrier and reach the interstitial space (77). In normal non-tumor tissues, vascular endothelial cells are tightly connected and have small para-cellular gaps in the 5–10 nm range. In contrast, larger gaps exist between endothelial cells in tumor blood vessels, ranging from 100 to 700 nm, depending on the cancer (78). The combination of the leaky tumor vasculature and the limited lymphatic drainage is called the enhanced permeability and retention (EPR) effect, which allows the passive disposition and accumulation of liposomes into the tumor site (79). In addition, these liposomes suffered from drug leakage during their systemic circulation (80). Changes were made to improve these liposomes, including composition and surface modification, to produce the second-generation liposomes. These liposomes had improved stability, disposition, and efficacy compared to first generation liposomes. Cholesterol was added in the lipid bilayers of liposomes to increase their rigidity and reduce drug leakage (81). The incorporation of polyethylene glycol or PEG (PEGylation) provided a steric protection of liposomes from electrostatic and hydrophobic interactions with plasma proteins, which decreased uptake by RES. In addition, PEGylation extended the circulation time of liposomes, allowing for a more effective drug delivery in vivo. These long-circulating liposomes were therefore named “stealth liposomes” (82). The first stealth liposomal formulation to be approved for cancer therapy in the United States (1995) and European Union (1996) was Doxil®/Caelyx® (83). Doxil® offers reduced cardiotoxicity and myelotoxicity in comparison to free doxorubicin, while achieving higher drug concentrations in tumors by using a liposomal composition of HSPC:CL:MPEG 2000-DSPE (calc. molar ratio 3:2:0.9, *w*/*w* 3:1:1). While the Doxil®/Caelyx® liposomal formulation is clinically efficacious, efforts have been made to change the formulation in order to improve the pharmacokinetic properties. Lipo-Dox® was created with a similar lipid molar ratio to Doxil®/Caelyx®

Although proven to be clinically useful, stealth liposomes depend mostly on their passive accumulation into tumor tissues; they lack the ability to control cellular uptake and drug release and rely only on passive drug efflux, which may result in limited efficacy. The third, or “new generation”, liposomes use ligand-mediated targeting or active targeting to improve biodistribution and liposome-mediated drug delivery at tumor sites (84).

Active Targeting of Liposomes

Passive targeting of liposomes relies only on the pathophysiological properties at the tumor site and has limitations that include decreased efficacy and/or off-target toxicity (85). One reason for this lack of clinical efficacy is that passively targeted liposomes lack true specificity for the tumor cells themselves. This has led several researchers to focus on more precise forms of targeting liposomes, such as active targeting. Active targeting uses molecular approaches to directly target tumor cells via interactions with tumor-specific markers (86). Actively targeted liposomes are usually prepared by conjugating targeting moieties such as monoclonal antibodies, fragments of antibodies, or peptides to their surface (87). This approach is a promising strategy for cancer therapy (88). Active targeting utilizes specific pathological changes in the tumor microenvironment such as the overexpression of several proteins. Therefore, liposomes targeting these markers can be selectively taken up by cells that overexpress these proteins to achieve improved drug delivery (89). While active targeting has the ability to target cells once liposomes are in the tumor microenvironment, it actually has no tumor targeting ability.

Antibodies are usually conjugated to PEG, and not to the liposomal phospholipids, to overcome the steric hindrance possibly caused by PEG interference with antibody-target protein interactions. Thus, the ligand is extended outside the PEG layer and is more accessible for binding to its target (90).

Local Stimuli to Trigger Drug Release from Liposomes

Strategies also exist to increase drug release from liposomes after they accumulate in the tumor. Many of these strategies take advantage of pathological changes in the tumor microenvironment, such as altered pH, increased temperature, and overexpression of proteolytic enzymes such as secretory phospholipases (91). External stimuli can also be applied to enhance or trigger drug release from liposomes (92). pH-sensitive co-polymers can be added in liposomal formulations that are stable at a physiological pH, but these will be hydrolyzed at an acidic pH of 6 and lower, which is commonly found in the tumor microenvironment. Taking advantage of specific pathological changes in the tumor microenvironment can increase drug release from liposomes, such as the overexpression of enzymes (e.g., matrix metalloproteinases (MMPs) and phospholipase A_2) (93). The activity of these enzymes can mediate the uptake and release of encapsulated drugs from enzyme-sensitive or responsive liposomes (94). Despite the extensive research and the development of different liposome formulations, the sub-optimal potency is still a major limitation of liposomes. For instance, the most successful nanomedicine, Doxil®, can only achieve modest benefits. Additional work is needed and has to focus on how to improve the therapeutic efficacy of liposomes. While these strategies exist to increase drug delivery, another factor that is limiting drug release includes the PEG layer in stealth liposomes (95).

PEGylation of Liposomes

PEGylation offers stealth properties to liposomes, including evasion of the mononuclear phagocytic system and extended circulation times that are responsive to PEG length and density (96). Increasing the percent of grafted PEG on liposomes (i.e., 2–5 mol%), and using PEG_{2000} or PEG_{5000}, markedly reduces protein adsorption, phagocytosis, and cellular adhesion of erythrocytes, lymphocytes, and macrophages (97). However, some of the beneficial properties of

PEGylated liposomes can create a few challenges for maximizing drug delivery, cell uptake, and endosomal escape.

Accelerated Blood Clearance

In animal models, increased blood clearance and increased accumulation in the liver and spleen can occur after a second injection (i.e., <4 weeks from the 1st injection) of PEGylated liposomes, which is known as the accelerated blood clearance phenomenon (98). In addition, the second injection (i.e. <1 week) of PEGylated liposomes result in significantly increased IgM production in rats (99).

Cell Uptake and Cargo Delivery of PEGylated Nanoparticles

PEG length and density play an essential role in cell uptake and endosomal escape. Starting simply on the level of measuring how PEGylation impacts liposome-to-liposome fusion, Holland et al. performed in vitro fusion assays measuring the changes in resonance energy transfer from mixing fluorescently labeled liposomes (i.e., Rh-PE and NBD-PE) with non-fluorescent liposomes, followed by the addition of $CaCl_2$ to promote liposome fusion(100).

In the area of liposome-based chemotherapeutics, PEGylation has offered reduced accumulation in the liver, spleen, and heart over free drug, but some studies report that tumor-targeting efficiency ($T_e = AUC_{tumor}/AUC_{plasma}$) is reduced in comparison to non-PEGylated liposomal formulations.

7.3. Cleavable PEG Coatings

In an effort to still use the steric stabilization offered by PEGylation, others have taken the approach to create cleavable PEG-lipid linkages in order to shed the PEG coating. One effective strategy to overcome these limitations is by installing acid-labile acetal, hydrazine, hydrazone, or vinyl ether linkages (101) between PEG and the lipid or polymer, which takes advantage of the slightly acidic tumor microenvironment (pH 5.6–7) (102) as well as endosomes (pH 5.5–6.5) (103). On the other hand, the liposomes with covalently linked PEG remained as a punctate distribution. Strategies to target liposomes (e.g., peptide-targeted liposomes) or use fusogenic materials to increase tumor cell uptake and drug delivery can be concealed with a cleavable PEG polymeric layer that would be shed upon an external stimulus (i.e., lower pH or reducing conditions) (104) and expose the targeting ligands to guide the liposomes to the tumor cells.

Overall, there is promising potential in achieving a balance between the stealth properties of PEGylated liposomes and maximizing drug delivery by shedding the PEG layer.

CONCLUSION:

Liposomes represent an attractive delivery system due to their physicochemical properties that allow overcoming various challenges and limitations with drug delivery. The use of liposomes to improve drug delivery has greatly impacted various biomedical areas. Liposomes have been shown to improve stability and biodistribution of therapeutic agents, overcome limitations to tissue and cellular uptake in target sites in vivo, and reduce systemic toxicity associated with non-encapsulated agents. However, despite the considerable preclinical work on liposomes, their translation into the clinic has progressed only incrementally. Future research will need to focus on addressing such translational limitations. This will require continuous communications and collaborations between experts in all stages of pharmaceutical development, including pre-clinical and clinical applications as well as toxicological evaluations.

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