Advances in Stimuli-Responsive Liposomes for Antitumour Drug Delivery Systems

Chen Zhu*

Wuya College of innovation, Shenyang Pharmaceutical University, Shenyang, China

*Corresponding author: zc123313@ldy.edu.rs

Abstract. Cancer, a disease caused by the abnormal proliferation and spread of cells, is responsible for nearly 10 million deaths in 2020, accounting for 1/6 of all deaths worldwide. The pathogenesis of cancer is complex and is usually triggered by a combination of genetic mutations, environmental factors and lifestyle. Current treatments such as surgery, chemotherapy and radiotherapy are effective but have significant side effects such as fatigue, infection and pain. As a drug delivery system, liposomes have the advantages of drug protection, delayed release, and targeted therapy, which can effectively reduce the toxicity of chemotherapeutic drugs. Liposome-encapsulated anticancer drugs, such as Doxil, DaunoXome, and Onivyde, have demonstrated promising efficacy in the treatment of a variety of cancers, such as breast and ovarian cancers, by prolonging the duration of action of the drugs and reducing side effects. By virtue of their unique structure and function, liposomes have improved the targeting and stability of cancer drugs and provided a new direction for anticancer therapy. Therefore, we have further explored the research progress of liposomes by investigating their properties, synthesis methods, classification and use in cancer therapy.

Keywords: Stimuli-responsive liposomes; Cancer; Delivery; Nanoparticle.

1. Introduction

Lung cancer, breast cancer, liver cancer, colorectal cancer, etc. is a group of diseases caused by abnormal cell proliferation and spread. Every year, millions of new cases of cancer are diagnosed worldwide, and the number keeps growing. Nearly 10 million people died of cancer in 2020 alone, making up one-sixth of all fatalities worldwide, making it the second most common cause of death worldwide, according to the World Health Organization [1]. The pathogenesis of cancer is complex and varied and is usually triggered by a combination of genetic mutations, environmental factors and lifestyle. Mutations in oncogenes and tumor suppressor genes, defects in DNA damage repair mechanisms, inflammation, viral infections, and exposure to carcinogens (such as tobacco and certain chemicals) are all common triggers for the occurrence of cancer. These factors allow normal cells to escape the regulatory mechanisms within the body, leading to the formation of malignant tumors. Moreover, a major contributing element to cancer's lethality is its ability to travel via the lymphatic or circulatory systems to other areas of the body. Surgery, chemotherapy, radiation therapy, immunotherapy, and targeted therapy are currently the mainstays of cancer treatment. But these techniques frequently result in negative side effects like exhaustion, hair loss, infections, discomfort, and vomiting [2].

Liposomes are a type of nanocarrier composed of a phospholipid bilayer. Due to their unique physicochemical properties, liposome technology has currently been used in various drug delivery systems for cancer treatments. The most typical example is Doxil, which is the first liposome-based anticancer drug approved by the FDA. By encapsulating doxorubicin in liposomes, Doxil can significantly reduce cardiac toxicity and enhance the accumulation of the drug at the tumor site. Other liposomal drugs such as DaunoXome and Onivyde have also demonstrated good clinical efficacy in various types of cancer [3]. Currently, stimulus-responsive liposomes are attracting a lot of attention from researchers because they can release encapsulated drugs under specific external or internal stimuli, significantly enhancing the drug delivery efficiency of liposomes.

In summary, liposomes, as an emerging drug delivery technology, not only effectively address the toxicity issues of traditional chemotherapy drugs but also offer new possibilities for cancer treatment. This article reviews the construction strategies, response mechanisms, clinical applications, challenges faced, and future prospects of liposomes, aiming to provide a reference for the research and application of liposomes in anti-tumor drugs.

2. Liposomes

2.1. The Basic Characteristics of Liposomes

Liposomes are vesicles typically ranging in diameter from 20 to 200 nm, composed of a phospholipid bilayer. Due to their unique double-layer structure, liposomes can encapsulate both hydrophilic and lipophilic molecules, which is why they are widely used in the field of drug delivery. Natural or synthetically produced phospholipids, such as phosphatidylcholine and phosphatidylethanolamine, are the main components of liposomes. These substances primarily come from food oils and fats, and they possess better biodegradability.

The advantages of liposomes in drug delivery include: 1): It can effectively protect the encapsulated active substances, making them more stable and shielding them from damage by enzymes and the immune system. 2): Control the release rate of active substances to achieve sustained release and extend the effectiveness of the medication. 3): Accurate medication delivery is possible by controlling the release of active ingredients within liposomes using external stimuli (such as temperature, pH, light, and magnetic fields). 4): Specific modifications to liposome membranes can enable active drug delivery, particularly for targeted therapy at diseased sites.

2.2. Methods for the Preparation of Liposomes.

The structure, particle size, and *in vivo* distribution of liposomes are influenced by various preparation methods. Furthermore, every preparation process has benefits and drawbacks of its own because the principles and features of liposome production vary in different ways. Thin film dispersion, reverse phase evaporation, solvent injection, and freeze-drying are typical preparation techniques.

3. Stimulus-Responsive Liposomes

3.1. Endogenous Stimulus-Responsive Liposomes

3.1.1 Ph-Sensitive Liposomes

pH-sensitive liposomes can undergo structural changes in acidic environments (such as tumor microenvironments or sites of inflammation), thereby releasing the drug. Due to the fact that the pH level of tumor tissue is usually lower than that of normal tissue, pH-sensitive liposomes can precisely release drugs in specific lesion areas, reducing damage to normal tissue. Okubo discovered that liposomes containing pH-sensitive carbohydrate derivatives can selectively bind to antigen-presenting cells in the skin and promote the endosomal escape of antigen proteins through the membrane's pH-responsive disruptive properties, thereby triggering antigen-specific cellular immunity [4]. Chaudhari evaluated the antitumor effects and biodistribution of different pH Lipo formulations in tumor models. The study showed that PEGylated pH Lipo (PEG-pH Lipo) and ADN-conjugated pH Lipo (ADN-PEG-pH Lipo) exhibited significant advantages in inhibiting tumor growth [5].

pH-sensitive liposomes have shown significant effects as carriers for anti-tumor drugs, but further improvements are needed in how they accurately sense pH levels and control drug release in the tumor microenvironment. In the future, adaptability and responsiveness to different pH environments can be enhanced by developing more sensitive and specific membrane materials or intelligent liposome structures.

3.1.2 Redox-Sensitive Liposomes

Redox-sensitive liposomes are sensitive to reducing conditions in the tumor microenvironment, such as higher concentrations of glutathione, and release drugs in a reducing environment. By introducing reduction-sensitive bonds (such as disulfide bonds), liposomes can achieve targeted drug release in tumors or other specific pathological tissues. Feng synthesized a novel bone-targeting and CD44-targeting redox-sensitive polymer and applied it to the surface modification of liposomes [6]. Research shows that liposomes coated with a novel polymer (ALN−HA−SS−L) exhibit significantly better targeting in osteosarcoma cell models compared to other control groups. These liposomes accumulate at the tumor site more than those containing only HA or those with only redox sensitivity, indicating that the disulfide bonds (-SS-) are efficiently cleaved in tumor cells. Peng developed and manufactured multi-target redox-sensitive liposomes (Lip-SPG) modified with glucose and triphenylphosphine (TPP) [7]. The study results showed that this drug delivery system could significantly enhance the targeting of drugs in glioma cells and strengthen the anti-tumor effect of the medication and provide new ideas and methods for the treatment of glioma.

Redox-responsive drug delivery systems represent a new field in nanomedicine and hold great potential in clinical cancer treatment. In the future, by designing carriers that are more precisely responsive to redox conditions, we can enhance their sensitivity to the lower reduction potentials or specific redox enzymes present in the tumor microenvironment, thereby improving the spatiotemporal precision

3.1.3 Enzyme-Sensitive Liposomes

Enzyme-sensitive liposomes are a type of nanocarrier that can release drugs in response to the activity of specific enzymes in the body. The design of this type of liposome takes advantage of the overexpression or activity of certain enzymes under specific diseases or physiological conditions. For example, in tumor or inflammatory tissues, the levels of certain enzymes such as phospholipases and matrix metalloproteinases are higher. Enzyme-sensitive liposomes degrade or rupture upon contact with specific enzyme targets introduced into their structure, thereby releasing the drugs they carry. Fouladi found that in tumor tissues, high concentrations of matrix metalloproteinase-2 (MMP-2) can cleave junctions, removing polyethylene glycol (PEG) from the surface of liposomes. This causes the surface of the liposomes to expose galactose, which is then taken up by liver cancer cells [8]. The study by Mock compared the therapeutic effects of secretory phospholipase A2 (sPLA2) responsive liposomes (SPRL) with clinically used long-circulating stabilized liposomes (SSL) both *in vitro* and *in vivo*. Research has found that SPRL exhibits better therapeutic activity than SSL both *in vitro* and *in vivo*, particularly the SPRL-E formulation, which shows higher uptake levels in prostate cancer cells [9].

Using enzyme-sensitive liposomes for targeted therapy is a novel strategy in stimulus-responsive drug delivery systems. With the continuous advancement of tumor signaling pathways and biomarker technologies, as well as the in-depth exploration of enzyme-sensitive functional components, enzyme-sensitive liposomes are expected to bring new breakthroughs in the field of cancer treatment.

3.2. Exogenous Stimulus-Responsive Carrier

3.2.1 Thermo-Sensitive Liposomes

Thermosensitive liposomes are a type of nanocarrier that can respond to temperature changes and release drugs. The design of this type of liposome is usually based on certain materials undergoing structural changes at specific temperatures, making the liposome membrane more permeable or causing it to rupture, thereby releasing the drug contained within. They are commonly used in cancer treatment because tumor tissue can be heated through external means (such as local heating) to activate drug release. Specifically, liposomes can release drugs when heated to a certain temperature (usually between 41-45°C), while remaining stable at normal body temperature (37°C). Moreover, through localized heating, the medication can be concentrated and released in the affected area, reducing systemic side effects. Additionally, thermosensitive liposomes can be combined with

temperature control technologies (such as ultrasound, radiofrequency, etc.) to activate drug release through non-invasive methods. Mo developed a polymer-based high thermosensitive liposome system (P-TTSL), where the most important polymer is p(NIPAM-r-HPMA), which can significantly enhance thermosensitivity when inserted into the lipid bilayer membrane. In *in vitro* experiments at 37℃ and 42℃, the liposome system exhibited a strong temperature dependence. Especially at 42°C, the system can achieve rapid release of doxorubicin (DOX) through thermal triggering, fully utilizing the concentration gradient of DOX in the tumor area [10]. This technology has shown great potential in preclinical studies and is expected to be widely used in cancer treatment in the future.

3.2.2 Photosensitive Liposomes

Photosensitive liposomes are a type of nanocarrier that can release drugs under specific light conditions. This type of liposome incorporates photosensitive materials or photosensitizers into its structure. When the liposome is exposed to light of a specific wavelength, the photosensitive materials undergo a photochemical reaction, leading to the disruption or alteration of the liposomal membrane structure, thereby releasing the encapsulated drug. Specifically, controlling the release of drugs from liposomes through external light sources (such as lasers or light of specific wavelengths) allows for precise spatiotemporal control. By concentrating the light source on the lesion area, the drug can be effectively released at a specific site, reducing the impact on healthy tissue and minimizing systemic side effects. It is worth mentioning that this type of liposome can activate drug release without invasive methods; photosensitive liposomes can achieve non-invasive treatment by integrating technologies such as photodynamic therapy. A research designed and synthesized five photosensitive azobenzene derivatives, finding that positively charged and strongly electrophilic substituents significantly reduced the photoisomerization ratio in the liposome system [11]. Additionally, the group can precisely regulate the rate of light-induced drug release by introducing different substituents at the 4′position of azobenzene-cholesterol derivatives. Developing a light-sensitive liposome drug delivery system (DDS) is very promising, as light possesses clean characteristics, non-invasiveness, and high efficiency. In addition, another important advantage of this technology is the ability to precisely control drug release by adjusting the duration and intensity of light exposure, which gives it broad application prospects in modern precision medicine.

3.2.3 Magnetic Field-Responsive Liposomes

Magnetic field-responsive liposomes are a type of nanocarrier system that can respond to external magnetic fields. This type of liposome can undergo physical changes and release the encapsulated drugs when an external magnetic field is applied, by embedding magnetic nanoparticles (such as Fe_3O_4 and Fe_2O_3 particles) into its structure [12]. The principle is that an external magnetic field can induce changes in the membrane structure of liposomes, promoting drug release or targeted accumulation in specific areas. Since the presence of magnetic particles, liposomes can be guided to specific areas within the body through an external magnetic field, achieving precise drug delivery and reducing the impact on healthy tissues. Therefore, Shinkai developed magnetic cationic liposomes (MCL) by encapsulating 10 nm magnetite nanoparticles in cationic liposomes [13].

Magnetic field-responsive liposomes have broad application prospects in cancer treatment, targeted drug delivery, and imaging diagnosis. By positioning the magnetic field at the tumor site, the drugs in the liposomes can be released in a concentrated manner, enhancing efficacy while reducing side effects. This technology combines nanotechnology and magnetic field control, enabling precise regulation of drug release, particularly suitable for treatment plans that require high targeting and controlled release.

4. The Current Status of Liposomes in Cancer Treatment.

The most common ways to treat cancer include surgery, chemotherapy, radiation therapy, and hormone therapy. However, these approaches frequently have unfavorable side effects, like pain, exhaustion, hair loss, infections, and vomiting. To enhance cancer treatments, a number of drug

delivery systems (DDS) have been created recently. DDS is a nanoplatform that can be used to carry medications to the affected areas and release their contents in response to external stimuli like light, temperature, mechanical waves, electric fields, and magnetic fields, as well as internal stimuli like pH, temperature, and enzymes. Various DDS are being created at the moment, including as carbon nanotubes (CNT), solid nanoparticles, silica nanoparticles, liposomes, dendrimers, and micelles. Among them, liposomes are one of the most effective DDS and have been widely utilized in targeted drug delivery.

The research on utilizing liposomes as carriers to encapsulate anticancer drugs began in 1973, aiming to deliver specific medications to tumors and address challenges in cancer treatment, such as toxicity, drug resistance, and non-specific tissue accumulation. Moreover, drug-loaded liposomes can enhance efficacy and prolong circulation time.

4.1. Doxil

Doxil is the first liposomal drug ever approved by the U.S. Food and Drug Administration (FDA), entering the U.S. market in 1995. It has been authorized to treat multiple myeloma, breast cancer and ovarian cancer. Doxil uses polyethylene glycol (PEG) modified liposomes, allowing it to evade the body's immune system, thereby extending its half-life in the bloodstream and enhancing its therapeutic effects [3]. Its success has also provided important experience for the development of other liposomal drugs, promoting the widespread application of liposomal technology in the field of drug delivery.

4.2. DaunoXome

DaunoXome is a citrate DNR liposome formulation, a product free from bacteria, pyrogens, and preservatives, intended for intravenous infusion. The American company NeXstar Pharmaceuticals developed DaunoXome in 1996 for the treatment of HIV-related Kaposi's sarcoma [3]. DaunoXome is another successful case of liposomal drug delivery technology in cancer treatment. By encapsulating daunorubicin in liposomes, it prolongs the drug's circulation time in the bloodstream, increases its concentration at the tumor site, and reduces toxicity to normal tissues. Like Doxil, the success of DaunoXome further confirms the advantages of liposomal technology in enhancing the efficacy of anticancer drugs and reducing side effects, providing strong support for the application of liposomal drug delivery systems in cancer treatment.

4.3. ThermoDox

ThermoDox is a heat-sensitive liposomal drug that contains doxorubicin and releases it upon local heating (40-45℃). The drug is designed to be used in conjunction with local hyperthermia therapy for tumor treatment, such as radiofrequency ablation (RFA) technology. By locally raising the temperature, the liposomes rupture, allowing for efficient release of the drug at the tumor site. The Phase III clinical trial named "OPTIMA" evaluated its efficacy in patients with liver cancer, but the results of this clinical trial were not satisfactory [14]. Future research needs to have a better understanding of the biophysical mechanisms of such formulations, which will help achieve greater success in the future.

4.4. SP1049C

SP1049C is an experimental anti-cancer drug that delivers doxorubicin through liposome technology and incorporates two non-ionic surfactants in its formulation, aimed at overcoming multidrug resistance (MDR) in tumors. Although SP1049C is not strictly a pH-responsive drug, its design takes into account the acidic conditions in the tumor microenvironment, which can enhance the drug's targeting ability in the tumor area to some extent. By combining liposome structure and surface modification, the accumulation and release efficiency of SP1049C in tumors has been improved. SP1049C has undergone multiple clinical trials in patients with gastric cancer and esophageal cancer, showing promising efficacy [15].

5. Challenges and Prospects

Although liposomes can improve drug accumulation at tumor sites, their targeting ability remains limited. Currently, liposomal drugs mainly rely on passive targeting, which accumulates at tumor sites through the enhanced permeability and retention effect (EPR). However, many tumor vascular structures are imperfect and may not be able to effectively achieve this effect. In addition, passive targeting cannot guarantee that liposomes will enter the interior of tumor cells, which may reduce their efficacy. Moreover, liposome technology can encapsulate drugs, but the release rate of the drugs is difficult to control precisely. Releasing the drugs at inappropriate times or locations may lead to decreased efficacy or increased side effects, especially for medications that require long-term action or precise delivery. Liposomes may also be recognized and cleared by the immune system, especially when unmodified liposomes are used. Although the use of PEG modification can extend their circulation time in the body, PEGylation may lead to immune reactions or the "accelerated blood clearance" phenomenon (ABC effect), which can affect the therapeutic.

One of the future research directions is to develop actively targeted liposomes by surface modification to target tumor-specific antigens or receptors, such as enhancing the targeting ability of liposomes through the conjugation of antibodies, peptides, or other targeting molecules. In addition, developing liposomes with controlled release characteristics can gradually release drugs according to therapeutic needs, thereby enhancing treatment effectiveness. The future development trends of liposome technology also include encapsulating multiple drugs, gene therapies, or immunomodulatory molecules within the same carrier for combination therapy. This strategy can enhance therapeutic efficacy and reduce drug resistance through multi-target action. In summary, liposome technology has broad application prospects in the field of anticancer drug delivery. With future technological advancements, it is expected to address many current limitations and provide more efficient and safer treatment options.

6. Conclusion

Liposomes are a type of nanocarrier composed of a phospholipid bilayer that can encapsulate and protect drugs, effectively reducing side effects. This article provides a detailed introduction to the working mechanisms and advantages of different types of stimulus-responsive liposomes, which have shown significant effects in tumor-targeted therapy. The significance of liposome technology lies in its effective enhancement of the targeting and safety of anticancer drugs, reducing the toxicity of traditional chemotherapy agents. In the future, with the development of smart responsive liposomes and the application of multi-drug combination therapies, liposome technology is expected to further enhance the precision and efficacy of cancer treatment, becoming a significant breakthrough in the field of cancer therapy.

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