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PH-SENSITIVE LIPOSOMES AND APPLICATION

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ABSTRACT

Liposomes have an aqueous, drug-containing interior surrounded by an exterior lipid bilayer, and typically range in size from 0.5 to 100 µm. Liposomes have been used successfully to reduce side effects of antitumor drugs and antibiotics. The Classification of liposomes Based on composition and size. PH-sensitive liposomes are designed to specifically triggered release the loaded drugs in response to the change of pH in the surrounding serum. So pH-sensitive liposomes can effectively deliver drug or gene fragments into the cytoplasm via the endocytotic pathway. Furthermore, pH-sensitive liposomes can be successfully used in clinical if they enable the encapsulated drugs to be targeted to pathological tissues (such as primary tumors, metastases, local ischemia, inflammation and infection) of the body in which pH is less than the normal physiological value. That is the reason why a growing amount of literatures described the development and applications of pH-sensitive liposomes to improve the therapeutic index of the encapsulated active ingredients. In this review, the commonly used pH-sensitive molecules for pH-sensitive liposome and the mechanisms of intracellular delivery of pH-sensitive liposomes were addressed. Besides, the potential clinical applications were fully discussed in detail with an expectation to contribute to the clinical research of pH-sensitive liposomes.

1. INTRODUCTION

1.1. Liposomes

Liposomes have an aqueous, drug-containing interior surrounded by an exterior lipid bilayer, and typically range in size from 0.5 to 100 µm. Liposomes have been used successfully to reduce side effects of antitumor drugs and antibiotics. For example, doxorubicin liposomes have reduced cardio toxicity and emetic side effects. Amphotericin B may have reduced nephrotoxicity side effects when formulated with liposomes. The lipid drug complex releases the drug at the site of infection and reduces renal toxicity of amphotericin B without altering its antifungal activity. Daunorubicin citrate liposomal is an aqueous solution of the citrate salt of the antineoplastic daunorubicin encapsulated within lipid vesicles.[1] The pharmacokinetics of liposomal doxorubicin are significantly different from those of the conventional drug formulation, with a decreased uptake by normal tissues (although tumour neovasculature is reported to have increased permeability to the liposomes), and a terminal half-life of 4 to 5 hours.[12] There are three general ways of preparing conventional liposomes: (1) Phase separation, (2) Spray or shear method through orifice, and (3) Coacervation. The choice of method depends on the drug, the yield requirements, and the nature of the lipids. Formation of the liposome bilayer depends on the hydrophobic and hydrophilic orientation of the lipids. Liposomes have different electrical surface charges depending on the type of material used. Liposomes can be engineered to be site specific. Generally, site specificity is conferred by the type of lipid or by inclusion of a targeting agent, such as a monoclonal antibody, into the liposome bilayer. Lipids or fusogenic peptides that facilitate membrane fusion, such as phosphatidyl ethanolamine or GALA and KALA peptides, respectively, have been used to improve liposome intracellular delivery.[1]

1.2. Classification of liposomes

1.2.1. Based on composition

Liposomes are composed of natural and/or synthetic lipids (phospho- and sphingo-lipids), and may contain other bilayer constituents such as cholesterol and hydrophilic polymer conjugated lipids. The net physicochemical properties of the lipids composing the liposomes, such as membrane fluidity, charge density, steric hindrance, and permeability, determine liposomes' interactions with blood components and other tissues after systemic administration. The nature and extent of liposome-cell interaction in turn determines the mode of intracellular delivery of drugs. Thus, the predominant mechanism behind intracellular delivery of drugs by liposomes may mainly depend on their composition, as

depicted in Fig. 2. Liposomes can be classified in terms of composition and mechanism of intracellular delivery INTO five types as: (i) Conventional liposomes (CL); (ii) PH-sensitive liposomes; (iii) Cationic liposomes; (iv) Immune-liposomes; and (v) Long-circulating liposomes (LCL).[2]

1.2.2. Based on size

The liposome size can range from very small (0.025 /µm) to large (2.5 /µm) vesicles. Furthermore, liposomes may have single or multiple bilayer membranes (Fig. 1). The vesicle size is a critical parameter in determining circulation half-life of liposomes, and both size and number of bilayers influence the extent of drug encapsulation in the liposomes. Based on their size and number of bilayers liposomes can also be classified into one of three categories: (i) Multilamellar vesicles (MLV); (ii) Large unilamellar vesicles (LUV); and (iii) Small unilamellar vesicles (SUV).[2]

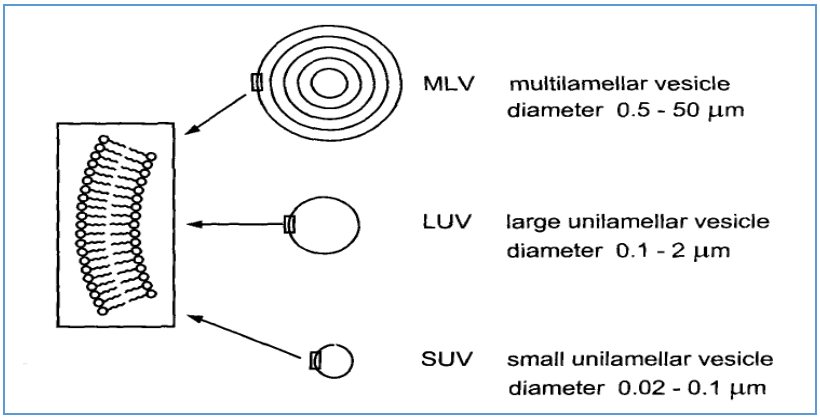


Fig. 1: Types of liposomes (Vesicle) depending on size.[11]

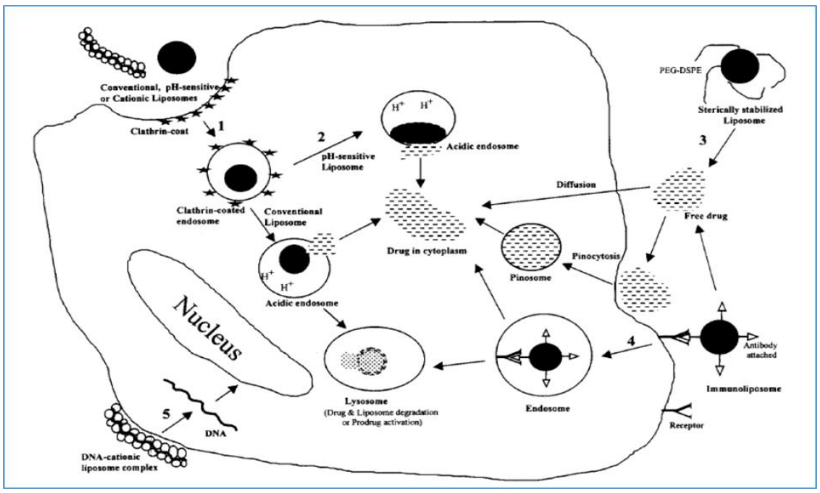


Fig. 2: Predominant mechanisms of intracellular drug delivery by liposomes. Numbers indicates the different pathways: l-coated pit endocytosis of conventional, pH-sensitive

and cationic liposomes; 2-release of drug in the acidic endosome by pH-sensitive liposomes; 3-intravascular and/or extracellular drug release from long circulating liposomes; 4-receptor mediated endocytosis of immune-liposomes; 5-fusion of cationic liposomes with plasma membrane.[2]

1.3. Physicochemical Properties

The physicochemical properties of the liposome drug product are critical to ensuring drug product quality. Therefore, a detailed evaluation of these properties should be provided. Rigorous characterization of the physicochemical properties can also be beneficial in evaluating subsequent changes in manufacturing. The physicochemical characterization tests, which are critical to ensuring product quality of each batch of liposome drug product, should be identified. However, all the characterization tests need not be included in the specifications. Properties specific to liposome drug products that may be useful to assess include: Morphology of the liposome, including lamellarity determination, if applicable: (1) Net charge, (2) Volume of entrapment in liposomal vesicles, (3) Particle size (mean and distribution profile), (4) Phase transition temperature, (5) Spectroscopic data, as applicable, (6) In vitro release of the drug substance from the liposome drug product, (7) Osmotic properties, and (8) Light scattering index.[3]

2. PH-sensitive liposomes

The concept of pH-sensitive liposomes emerged from the observation that certain enveloped viruses infect cells following acidification of the endosomal lumen to infect cells, and from the knowledge that some pathological tissues (tumors, inflamed, and infected tissue) have a more acidic environment compared to normal tissues. Although pH-sensitive liposomes are stable at physiological pH, they destabilize under acidic conditions, leading to the release of their aqueous contents. In addition, they appear to destabilize or fuse with the membranes of endosomes in which they are internalized, enabling even macromolecular liposome contents to enter the cytoplasm. The response to acidic pH can be facilitated by a variety of molecules, including fusogenic peptides incorporated in the lipid bilayer, pH-sensitive lipids and pH-sensitive polymers on the surface of liposomes. The combination of phosphatidylethanolamine (PE) or its derivatives with molecules with a protonatable group (e.g., carboxylic group) that acts, as a stabilizer of PE membranes at neutral pH, is the most commonly used composition. PE has a minimally hydrated and small head group that occupies a lower volume compared to the hydrocarbon chains, and can be imagined to have a

cone shape, in contrast to the cylinder shape exhibited by phospholipids such a phosphatidylcholine (PC). Strong intermolecular interactions between the amino and phosphate groups of neighboring polar head groups, along with the cone shape, facilitate the formation of an inverted hexagonal phase at temperatures above a critical temperature (TH) characteristic of the species of PE. These properties preclude the preparation of liposomes composed solely of PE or its derivatives under physiological conditions of pH, ionic strength, and temperature.[4]

2.1. Classification of pH-sensitive liposomes

Four basic classes of pH-sensitive liposomes have been described previously. The first class combines polymorphic lipids, such as unsaturated phosphatidylethanolamines, with mild acidic amphiphiles that act as stabilizers at neutral PH. This class of pH-sensitive liposomes has been the most intensively studied. The second class includes liposomes composed of lipid derivatives resulting in increased permeability to encapsulate d solutes. A third class of pH-sensitive liposomes utilizes pH-sensitive peptides or reconstituted fusion proteins to destabilize membranes at low ph. The final and most current class of pH-sensitive liposomes uses pH-titratable polymers to destabilize membranes following change of the polymer conformation at low ph.[5]

2.2. Triggered Release

Considering the multitude of factors that might cause biological destabilization of liposomes it may seem to be a simple task to obtain drug release from liposomes.[6]

2.2.1. PH-triggered release

In many applications, the liposome-encapsulated drug needs to be delivered to a specific site but as long as the drug remains, trapped inside the liposomes, it stays inactive. A slow drug release is, in most cases, not sufficient for an efficient treatment. Different types of liposomes, such as temperature- and pH-sensitive liposomes, have been developed for this purpose. The basic idea is that an environmental change will trigger the liposomal membrane to structural rearrangements that induce a leakage of the encapsulated substance. The aim was to increase the understanding of triggered release from pH-sensitive liposome systems, and more specifically to discriminate between the different release mechanisms possible for a cytosolic drug delivery. The use of PEG-lipids as stabilizers of DOPE liposomes serves dual purposes: liposome formation is facilitated and at the same time, the PEG-lipids provide steric stabilization. However, even small amounts of PEG-lipids in the DOPE membrane

prevent LI-HII transition at low pH, i.e. no triggered release is achieved. If the PEG is attached to the lipid by an acid-labile linkage, the cleavage and loss of the PEG moieties accompanying a pH reduction restore the pH-sensitivity of the liposomes and an LI-HII transition is made possible. A schematic representation of a pH-triggered release. **Figure (3)**. Mildly acidic amphiphiles, such as oleic acid (OA) and cholesteryl hemisuccinate (CHEMS), are other stabilizers that are commonly used in triggered release systems of DOPE liposomes.[6]

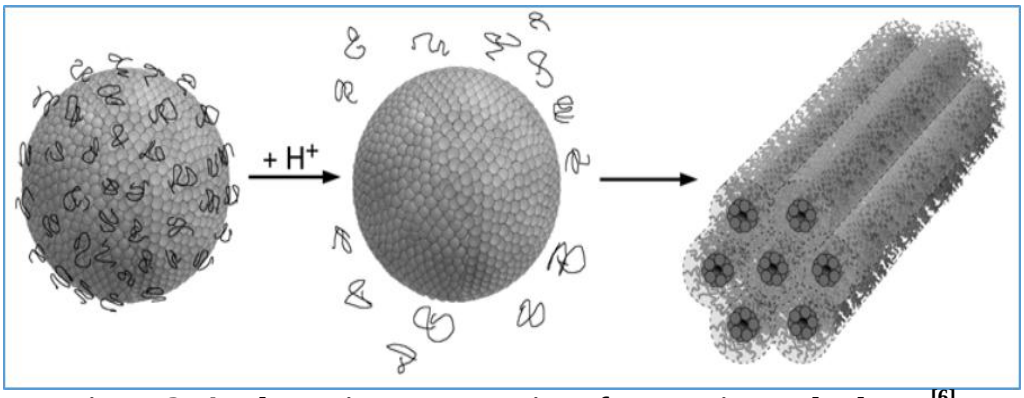


Figure 3: A schematic representation of a pH-triggered release.[6]

By incorporation of the conformational switchable, trans-2-morpholinocyclohexanol - based amphiphile (flipid) into lipid compositions, we designed pH-trigger-able liposomes (fliposomes) with extraordinary characteristics: high stability in storage and in serum in vitro combined with instant release of their cargo in response to a weakly acidic medium. The acid-induced release from fliposomes can be modulated by the mole percentage of the flipid, and is independent of inter-liposome contact. Based on fluorometric, NMR and electron microscopy studies, a mechanism of pH-sensitivity has been proposed that starts with an acid-triggered conformational flipid and is followed by a set of membrane perturbations, which cause the leakage. Anticancer activities of selected methotrexate formulations in HeLa cells demonstrate that fliposomes can serve as viable drug delivery systems.[7]

2.3. PH-sensitive liposomes: Acid-induced liposome fusion

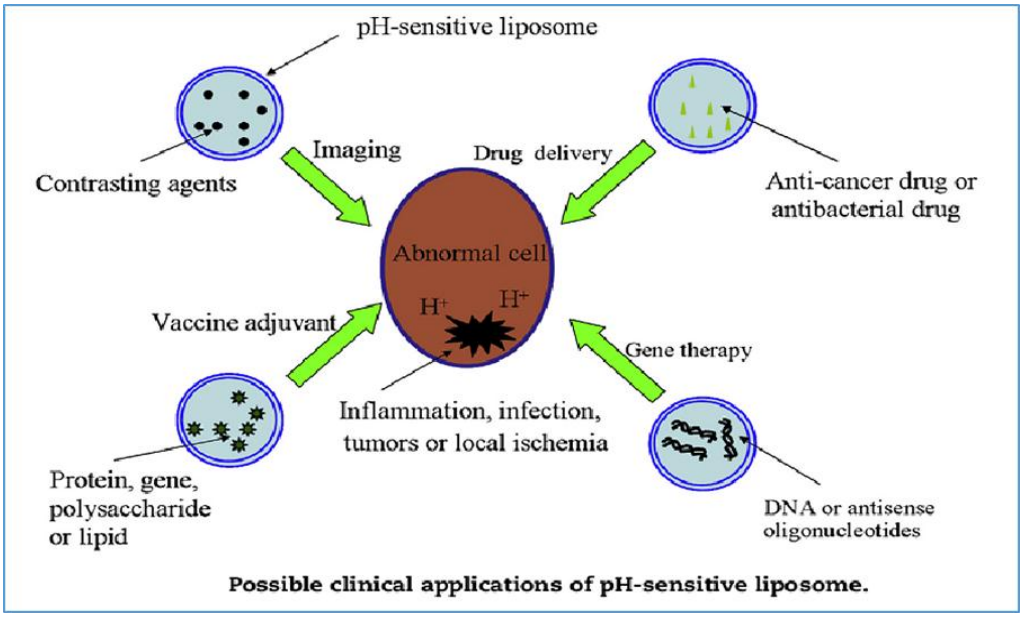
Proteins do not mediate the acid-induced liposome fusion or other macromolecules has not been demonstrated previously. In the case of liposome fusion mediated by proteins-e.g., serum albumin, clathrin, and viral glycoproteins -it is clear that the protein conformation change is the primary driving force for fusion. The driving force comes from the lipid itself, most likely palmitoylhomocysteine (PamHcy). Homocysteine forms a thiolactone ring at acidic pH. This mechanism was thought to be responsible for the pH-sensitive leakage in

liposomes composed of disaturated phosphatidylcholine (PtdCho) and PamHcy. However, recent evidence argues against this mechanism. Rather the pH effect may be explained by a change in "acid-base" equilibrium i.e., the ratio of charged to uncharged N-acylamino acid with subsequent changes in the electrostatic interactions among the lipid head-groups. Alternatively, the bilayer solubility of the protonated PamHcy might be so low that domains of PamHcy are formed at acidic ph. Such lateral phase separation of the bilayer lipids could be a major cause of fusion. The latter mechanism appears particularly attractive because we have found that the presence of phosphatidylethanolamine (PtdEtn) greatly enhances the acid-induced liposome fusion. Pure unsaturated PtdEtn, such as dioleoyl PtdEtn used in this study, is prone to form hexagonal phase or inverted micelles, and this tendency has been proposed as a primary mechanism for membrane fusion in other systems. In either case, it would generate a profound structural alteration of the lipid bilayer. Furthermore, these mechanisms suggest that other long-chain amphiphiles containing carboxylic groups, such as fatty acids, can also be used for acid-induced liposome fusion. The interactions of liposomes with animal cells have been extensively studied. Although liposome-cell fusion was initially suggested as a primary mechanism, it is clear from the recent studies in this and other laboratories that the endocytosis of liposomes is primarily responsible for liposome uptake. Furthermore, Jerome C, et al. have shown that the endocytosed liposomes encounter an acidic environment once they enter the endosomes. The pH of endosomes has been determined to be about 5. Therefore, it is conceivable that the liposomes used in this study could provide an effective cytoplasmic delivery system by fusing with the endosome membranes. The less leaky liposomes containing cholesterol may be particularly useful in discharging their contents into the cytoplasm.[8]

3. Applications of the pH-sensitive liposomes

Various liposomal formulations of polyene antifungal drugs have been found to impart cure against most common fungal pathogens. The liposomal formulations increase the therapeutic index of the drug by delivering higher concentrations to the infected tissues and therefore reducing the toxicity of the drug to the normal cells. The use of pH-sensitive liposomes significantly enhanced intracellular delivery of nystatin and thus showed enhanced antifungal activity in terms of increased survival rate, as well as reduced fungal burden in brain and liver. Mice treated with pH-sensitive nystatin liposomes showed enhanced survival (~80%) compared with those treated with neutral egg-PC-nystatin liposomes (~50%) or free nystatin (~10%) at equivalent doses (5 mg/kg). Mice treated with nystatin in pH-sensitive liposomes

(3 mg/kg) showed survival (~50%) equal to those treated with nystatin in neutral liposomes (5 mg/ kg). This clearly supports that pH-sensitive liposomes deliver substantial amount of drug to sites of infection compared with egg-PC liposomes. Although neutral liposomes are also taken up by macrophages, their penetration into the endosomes is not as high as that of pH-sensitive liposomes. It has been demonstrated that pH-sensitive liposomes increase the efficacy of gentamicin against Salmonella typhimurium infection in mice. The dissociation of the drug from pH-sensitive liposomes and their rapid accumulation in the target organs (the liver and spleen) makes these pH-sensitive carriers ideal for in vivo evaluation in an antifungal as well as antibacterial efficacy model. The delivery of nystatin using pH-sensitive liposomes immediately after infection results in the destruction of the pathogen because the environment, which was favourable for the survival of the pathogen, is loaded with the antifungal nystatin. As a result, most of the infection is subsided owing to the use of pH-sensitive nystatin liposomes that release substantial drug at the pH favourable for C. neoformans. Use of pH-sensitive liposomes seems a good strategy to fight against intracellular pathogens, which are otherwise very difficult to eliminate from their shelter sites. It can be concluded that nystatin, toxic in free form, can be made more effective and safe by entrapping it within pH-sensitive liposomes for treatment of fungal diseases.[9] After being endocytosed in the intact form, pH-sensitive liposomes fuse with the endovacuolar membrane on the condition of lower pH value inside the endosome and destabilize it, thus releasing their content into the cytoplasm. So pH-sensitive liposomes can be appropriately designed to release their encapsulated contents, especially the biological macromolecules, such as drugs, enzymes, antibodies and antisense oligonucleotide (ODN), plasmids, proteins and peptides, into cytoplasm before reaching the lysosome to ensure the activity of drugs. Besides, inflammation, infection, some tumors and local ischemia all will lead to abnormal acidification of the pathological tissues, so pH-sensitive liposome in the pH range of 6.5～7.4, as a delivery carrier, has great clinical value. That's why different applications of pH-sensitive liposomes were envisaged, including for the transport and specific delivery of potent drugs (for cancer, pulmonary and infectious diseases), vaccination (as immunological adjuvants), imaging (carrying contrasting agents) and as well as nucleic acids which is aiming at gene therapy applications **(Fig.(4))**.[10]



Possible clinical applications of pH-sensitive liposome.

3.1. Drug delivery

PH-sensitive liposomes are stable at physiological pH (pH 7.4) but undergo destabilization, and acquire fusogenic properties under acidic conditions, thus leading to the release of their aqueous contents. Therefore, in theory, pH-sensitive liposomes can prolong the circulation time and improve the efficiency of drug delivery. In practical, pH-sensitive liposomes have been reported to have possible clinical implications for delivering drugs to target sites such as primary tumor and inflammation sites where the pH could be less than physiological.[10]

3.2. Anti-tumor therapy

The systemic chemotherapy is almost impossible to achieve therapeutic levels of a drug at the solid tumor without injuring the healthy organs and tissues. In addition, several drawbacks, such as low bioavailability of the chemo-toxin, low drug concentrations at the tumor site, lack of specificity and drug-resistant also provide obstacle to its clinical applications. Although the nano-carriers less than 200 nm are able to be passively targeted to tumor tissue due to the enhanced permeation and retention (EPR) effect. However, one of the drawbacks of the conventional drug delivery system is the fast elimination from the blood and capture by the cells of the Reticuloendothelial system (RES), primarily in the liver. It is reported that the extracellular environment of solid tumors is acidic with a pH ranging from 5.7 to 7.8 compared with the pH 7.4 of the blood and normal tissue. PH-sensitive liposomes can be induced to undergo a pH-induced fusion of liposomal membranes with endosomal membranes or destabilization of the endosomal membrane, thus releasing contents into cytoplasm. Since most liposomes are internalized by endocytosis, pH-sensitive liposomes

undergo destabilization at this step and thus prevent degradation at the lysosomal level, which can promote cytosolic delivery of the intact contents. In recent years, as a drug carrier, the research and application of pH-sensitive liposomes in the treatment of cancer develop rapidly. At present, DOPE may be the most commonly used lipid for pH-sensitive liposomes. In general, PEG served as a stabilizer of DOPE containing pH-sensitive liposomes for triggered release of the loaded anti-cancer drugs. Ishida et al prepared doxorubicin encapsulated pH-sensitive liposomes with the mixture of DOPE/HSPC / CHEMS / CHOL / mPEG2000-DSPE at a molar ratio of 4:2:2:2:0.3 and DOPE / HSPC / CHEMS / CHOL at a molar ratio of 4:2:2:2 in the hydration way. As a result, pH-sensitive liposomes increased intracellular drug release rates within acidic compartment, resulting in a further increase in the therapeutic efficacy of B lymphoma. Besides, the cisplatin loaded pH-sensitive liposomes with DOPE / CHEMS / DSPE-PEG were prepared to cure the small cell lung cancer. Compared with free cisplatin, this formulation has a better stability in blood and its cytotoxicity is significantly enhanced. Furthermore, it is effective for the cells that are tolerance to cisplatin. So the addition of lipids with covalently attached PEG in liposomes was shown to avoid the rapid identification and elimination of the MPS, which may contribute to fully take advantage of the superiority of pH-sensitive liposomes. PH-sensitive liposome modified with monoclonal antibody could be directed to target to the lesions with a low pH environment. The therapeutic efficacy of the anti-cancer drug entrapped in pH-sensitive liposomes can be improved by the monoclonal antibody that can direct the pH-sensitive liposomes to the cell surface receptors. Kim et al developed gemcitabine pH-sensitive liposome (DOPE and CHEMS) with epidermal growth factor receptor (EGFR) antibody attached and used A549 cells and BALB/c-nu/nu mouse tumor model for testing. The results showed that treatment of pH-sensitive immuno-liposomes encapsulating gemcitabine resulted in an increased apoptosis of tumor cells, leading to tumor growth inhibition. Simard and Leroux formulated pH-sensitive immuno-liposomes by including a terminally alkylated copolymer of NIPAM in the liposome bilayer and by coupling the anti-CD33 monoclonal antibody to target leukemic cells. Finally, the pH-sensitive ILs-CD33 formulation exhibited the highest cytotoxicity against HL-60 cells. Last but not the least, nucleic acid, plasmid DNA or anti-sense oligonucleotides mediated by pH-sensitive liposome can be delivered in the treatment of cancer, as well. The detailed significance of pH-sensitive liposomes will be discussed in the section of gene therapy.[10]

3.3. Anti-infection therapy

Intracellular infection by bacterial is difficult to manage clinically and is often refractory to conventional chemotherapeutic treatment strategies due to poor penetration of drug into cells. As one of the drug carriers mentioned above, liposomes have achieved their greatest success against facultative and obligate intracellular pathogens in the treatment of infectious diseases, most notably those with a tendency to infect the MPS. Furthermore, liposomes have shown a particular validity in the treatment of infections by intra-cellular bacteria. In case the infectious focus is located outside the MPS, conventional liposomes are of limited value. Therefore, research has been aimed at decreasing the MPS uptake of liposomes and consequently increasing their circulation time and targeted distribution. PH-sensitive liposomes can be triggered to release their contents and fuse with the bio-membrane in response to acidic environment of the infected and inflamed tissues. Therefore, the dissociation of the drug from pH-sensitive liposomes and their rapid accumulation in the target organs (the liver and spleen) makes pH-sensitive liposomes an ideal candidate for in vivo evaluation in an antifungal as well as antibacterial efficacy model. The encapsulated gentamicin into pH-sensitive liposomes composed of DOPE-N-succinyl-DOPE and DOPE-N-glutaryl-DOPE (70:30; mol: mol) to treat with murine macrophage-like J774A.1 cells those were infected with bacteria. As a result, gentamicin encapsulated in lipid vesicle that undergo pH-dependent lipid mixing and fusion confered to this membrane-impermeative antibiotic a significant improvement in therapeutic activity against intracellular bacterial infections. Evaluated the efficacy of pH-sensitive liposomes of nystatin against Cryptococcus neoformans infection in a murine model. As a result, pH-sensitive liposomes of nystatin showed better efficacy compared with its free or egg-PC liposome form against C. neoformans infection in BALB/c mice. So the enhanced anti-cryptococcal efficacy of the pH-sensitive nystatin liposomes can be attributed to the pH-dependent release of the drug in the low pH environment of lysosomes. Exploited the fusogenic properties of DOPE / DPPC / CHEMS unilamellar vesicles with the purpose of releasing the antibiotic not inside cells but specifically in the narrow area of periplasmic space of Gram-negative bacteria. As a result, the outer membrane barrier can be bypassed and antibiotic can operate its molecular activity at the level of the cell wall. The enhanced efficacy observed for encapsulated antibiotics in pH-sensitive liposomes may due to targeted delivery of lipid carriers to the infected area.[10]

3.4. Gene therapy

Somatic gene therapy has emerged as a new approach for the treatment of a variety of genetic and acquired diseases. The key to success for any gene therapy strategy is to design a vector that is able to serve as a safe and efficient gene delivery vehicle. At present, the common carrier for the study and clinical application of gene therapy includes viral vector and non-viral vector. The viral vector has the natural ability to infect cells efficiently, but there is a potential risk of generating an infectious, replication-competent virus during the production or use of viral vectors for gene transfection. While the non-viral vector has no immunogenicity and it is easily prepared, so it has higher safety in vivo. Liposomes based gene vector has been promoted as a means of achieving the transfection efficiency of viral vector without the associated risks. In recent years, with the advent of cationic liposome and active targeting technology, liposome technology has been widely applied in the transfer of antisense ODNs for its virtues of high transfection efficiency, protection for the entrapped and potential of chemical modification. Besides, they are noninfectious, non-immunogenic, simple, and easy to produce in large scale. However, non-viral vector, like cationic lipids/liposomes, also showed certain drawbacks, such as non-specificity and cytotoxic reactions. Besides, the efficiencies of gene transfection meditated by conventional liposomes were accordingly low. However, pH-sensitive liposomes can release the loaded gene expression system in the cytoplasm before entering the lysosome by fusion with the biofilm due to the lipid bilayers of the basic structure of their biofilm. That is why pH-sensitive liposomes can transfect gene into cytoplasm more efficiently and avoids lysosomal degradation to some extent. Therefore, the pH-sensitive liposomes may be a promising non-viral vector for gene therapy. Transfected of a murine DC2.4 cells with pH-sensitive fusogenic liposomes that comprise polymers based on poly (glycidol) with carboxyl group. The results indicated these complexes with pH-Sensitive fusogenic liposomes exhibited higher transfection activity toward DC2.4 cells than some commercial reagents and hence may be use-full as a gene vector for DCs. However, the transfection efficiency of gene delivery directly mediated by pH-sensitive liposomes was less than the cationic liposomes owning to the negative charge. So the pH-sensitive cationic liposomes are expected to be an excellent gene carrier. Pre-condensed plasmid DNA with an arginine-based cationic surfactant, arginine-N-lauroyl amide dihydrochloride (ALA), which was incorporated the blood protein transferrin (TF) into two cationic liposomal formulations. One composed of a mixture of dioleoyl trimethylammonio propane and cholesterol (DOTAP: Chol) and the other pH-sensitive formulation constituted of DOTAP, Chol, DOPE and CHEMS. The results

demonstrated complexes based on the pH-sensitive liposomal formulations present better transfection profiles. Although, compared with the non-pH-sensitive immuno-liposomes, pH-sensitive immuno-liposomes have much higher capacity to mediate cytosolic delivery of the encapsulated therapeutic molecules due to endosomal escape. Their stability in the presence of plasma proteins and the stability of obtaining a sustained-release of the therapeutic agent still put obstacles to their applications in gene therapy. PEG modification may be a very interesting strategy to solve the problems mentioned above. C-DOPE, a derivative of DOPE that hydrolyzes rapidly at pH 5 to yield DOPE, was synthesized by Low et al and incorporated with DOPE and folate-PEG-DOPE into liposomes. The resulting pH-sensitive liposomes were stable at neutral pH and had a higher transfection efficiency compared with DOPE-cholesterol hemi-succinate based vectors.[10]

3.5. Vaccine delivery adjuvant

Since 1974, firstly reported that liposomes can be used as immunological adjuvant; thorough study has been made on the implications as a vaccine carrier and adjuvant of liposomes. From disposition studies of liposome in vivo, it is reported that large liposomes are efficiently taken up by macrophages of RES in blood and tissues, including the liver and spleen (the main immune organs), which contributes to deliver the antigen to antigen-presenting cells or other immune cells. Furthermore, liposomes have the function of immunological adjuvants without the side effect of common adjuvant .A liposomal vaccine against hepatitis A successfully developed by the Swiss Serum Institute (Bern, Switzerland) provided the best proof. However, conventional liposomes are endocytosed on contact with antigen-presenting cells and degraded, coupled with the entrapped molecules, inside the endosome via endosome-lysosome pathway. Whereas, pH-sensitive liposomes release liposomal antigen into the cytoplasm after endocytosis because of their fusion capacity with the endosomal membrane at low pH (range from 5.5 - 6.5 in the early and late endosome compartment). Then they were trans-ported to the endoplasmic reticulum where they combined with class I molecules. So pH-sensitive liposomes can deliver the encapsulated material more safely and efficiently than conventional liposomes, which suggests pH-sensitive liposomes may be a superior vaccine delivery adjuvant. PH-sensitive liposomes have been used as a non-viral adjuvants with bacterial, viral, protozoan, tumor and other antigens. prepared and characterized the Carboxyl-terminal 19 kDa fragment of merozoite surface protein-1 of Plasmodium falciparum ($PfMSP\text{-}1_{19}$) encapsulated pH-sensitive liposomal formulations using oleyl alcohol (OAlc) in combination with EPC as the membrane destabilizing components. The

results demonstrated pH-sensitive liposomes showed excellent immuno-adjuvant action and enhanced the immunogenicity of a soluble malaria antigen. Therefore, the present study of pH-sensitive liposomes might open new ways for the feasibility for the development of blood stage malaria vaccine. Besides, the presentation of CTL-peptide antigen mediated by pH-sensitive liposomes occurs in lymph nodes. Investigated the antigen delivery route by pH-sensitive liposomes in vivo using fluorescein isothiocyanate (FITC)-conjugated H-2K^{b} cytotoxic T lymphocyte (CTL) epitope as a model system. The pH-sensitive liposomal formulations showed significant effects on the generation and activation of antigen specific CTLs, indicating that the formulations might be used as a potential peptide adjuvant for priming and boosting against target antigens. The results suggest that pH-sensitive liposomes, as a strong peptide adjuvant, may be useful for peptide delivery for the development of therapeutic or prophylactic vaccines. Furthermore, stronger cellular immune responses can be induced by ovalbumin-loaded pH-sensitive liposomes from nasal cavities of mice. Developed ovalbumin-encapsulated pH-sensitive liposomes modified with poly (glycidol) derivativessuc hassuccinylatedpoly (glycidol) and 3-methylglutarylated poly (glycidol). Such pH-sensitive liposomes were applied to DC2.4 cells, a murine dendritic cell line, to investigate the potential of this formulation as a carrier of anti-gen proteins for induction of cellular immunity. The results indicated that the ability of the polymer-modified pH-sensitive liposomes to activate cellular immunity and the feasibility to develop efficient vaccines for immunotherapy. Liposomes have been reported to promote immune responses to DNA vaccines by facilitating uptake of the plasmid by antigen-presenting cells. A mechanism-based development of a siRNA delivery system that was optimized for endsomal fusion by modifying on a lipid mixture with a pH-dependent fusogenic peptide (GALA). Furthermore, they applied this system to deliver siRNA to primary mouse bone marrow-derived dendritic cells. The results demonstrated that siRNA loaded in this system efficiently suppressed endogenous gene expression and consequently enhanced dendritic cell-based cancer vaccine in vivo.[10]

3.6. MRI contrast agents

Magnetic resonance imaging (MRI) technique has become one of the most important diagnosis tools available in medicine. A majority of MRI contrast agents in clinical used today are based on paramagnetic gadolinium complexes that shorten the relaxation times of free water protons. The contrast agents in combination with MRI have been effective tools to get a perspective of inflammation, infarct, tumor, atherosclerotic plaques, live stem-cell

tracking, brain perfusion and many other applications. Most of these MRI contrast agents are complexes of gadolinium (Gd III) as this kind of ion has a high magnetic moment and a long electronic relaxation time. They can effectively pass through the damaged blood-brain barrier and can be quickly excreted by renal. Therefore, their enhancement effect is not proportional with the concentration. These contrast agents are all toxic and non-specific, even if their distribution in the body is far from homogeneous. As a new contrast agent, liposomes have received growing attention because of relatively long circulation time in blood, the ability of development, easily controlled properties and good pharmacological characteristics. However, the contrast agent of conventional liposomes is easily ruptured and absorbed by RES, which may reduce the contrast effect. Finally, MRI contrast agents are presented that react to variables in their environment, such as magnetism and ph. This concept of pH-mediated drug release could be investigated in MRI of tumors, infection and local ischemia. Paramagnetic pH-sensitive liposomes accumulated in the acidic environment within the pathological tissues could be triggered to structural rearrangements and thus release the encapsulated contrast agents into the cytoplasm. Therefore, if properly designed, these pH-sensitive liposomes would exhibit a function as “off-on” switches and markedly increased contrast effect. Xin L et al assessed the in vitro potential of several paramagnetic complexes loaded pH-sensitive liposomes formulated with the fusogenic phospholipid POPE and the membrane stabilizer D-α-tocopherol-hemisuccinate, as imaging tools for visualizing drug delivery and release processes by MRI. It was found that the resulted pH-sensitive liposomal formulation has the potential for visualizing drug delivery and release processes by in vivo MRI. Besides, the basic properties of pH-sensitive liposomes loaded with MRI agents were investigated.[10]

4. Conclusion and future prospects

PH-sensitive liposomes can significantly increase cytoplasmic delivery of various fluorescent markers with various molecular sizes, ribozymes, enzymes, cytotoxic agents, proteins, RNA, and DNA to cells with considerable efficiency. However, so far, none of this kind of preparations is used in clinical due to their drawbacks. Because, a clinically viable pH-sensitive liposomal formulation requires several essential properties including efficient pH-triggered release, serum stability, and enough long circulation time in vivo. Additionally, after being injected into the body, pH-sensitive liposomes still can be recognized by the opsonin in the plasma and phagocytized by RES to some extent, which is an important limitation to the in vivo use and the main barrier of the delivery of drugs and gene to

pathological organs (in addition to the liver, spleen). Furthermore, the physicochemical and biological stability issues, acid sensitivity and bioavailability, particle size control, batch-to-batch reproducibility and sterilization method are still to be overcome in order to satisfy the prerequisites of treating diseases in animals or humans. While as novel, responsive polymer compositions are continually being developed and the ability to prepare macromolecules with topological complexity is expanding, those problems mentioned above will be solved gradually. By that time, pH-sensitive liposomes would have been an attractive carrier for therapeutic drugs or macromolecules with intracellular targets. Furthermore, developing 'smart' multifunctional pharmaceutical nano-carriers by combinating of pH sensitive liposomes with active targeting and other release mechanisms (such as enzyme-responsive, temperature sensitive, light-sensitive, magnetic responsive and ultrasound-responsive), and selecting appropriate pH sensitive compositions, triggering signal and mechanism of action to be suitable for a specific application, pH-sensitive liposomes could be utilized in numerous medical treatments for enhanced efficiency in the foreseeable future.

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