ABSTRACT

The demand for increased specificity of delivery of anticancer agents to target tumors has resulted in numerous strategies. Improved delivery of drug-carrying immunoliposomes is one approach to this problem. One effective means of targeting tumors is via conjugation of antitumor antibodies or portions of antibodies to liposomes (immunoliposomes). In this approach, many factors must be taken into consideration, including proper choice of target antigen, antibody function, and antibody-liposome linkage. Thus, effective targeted drug delivery using immunoliposomes requires considerations of liposome, antibody and the chemotherapeutic agent, as well as their interactions with each other and the targeted cell.

Immunoliposomes can overcome the potential barriers for delivery into tumor tissues, suggesting that with proper construction of the Fab’ fragment of a properly chosen monoclonal antibody, proper liposome composition and proper drug loading, immunoliposomes can be effective anticancer agents.

Key words: immunoliposomes, anticancer agents, immunoliposome delivery

SAŽETAK

Potreba za povećanjem specifičnosti dostave lijekova do ciljanih tumora je rezultiralo brojnim strategijama. Poboljšana dostava lijekova imunoliposomima je jedan od pristupa ovom problemu. Jedan učinkoviti način za ciljanost dostave lijekova do tumora je putem vezivanje antitumorskih antitijela ili dijelova antitijela na liposome (imunoliposomi). U ovom pristupu, mnogi faktori moraju biti uzeti u obzir u obzir, uključujući pravilan izbor ciljnog antigena, funkcija antitijela, te način vezivanja antitijela na liposome. Dakle, učinkovita ciljana isporuka lijekova putem imunoliposoma mora uzeti u obzir faktore vezane za liposome, antitijela i lijekove, kao i njihove interakcije jedni s drugima kao i sa ciljanim stanicama.

Imunoliposomi mogu prevladati potencijalne prepreke za isporuku u tumorska tkivija, što ukazuje da je uz pravilan izbor odgovarajućeg fragmenta antitijela, pravilan izbor lipopsomskog sastava kao i pravilan izbor i količinu lijeka, imunoliposomi mogu biti učinkovita sredstva za dostavu antitumorskih lijekova i povećanje njihove efikasnosti.

Ključne riječi: imunolipozomi, antikancer sredstva, dostava imunolipozoma

1. INTRODUCTION

The concept of drug targeting and controlled drug delivery is used in attempts to improve the therapeutic index of drugs by increasing their ability to target specific organs, tissues or cells and by decreasing their activity, quantity and potential toxic side effects, such as in the field of cancer chemotherapy. Because chemotherapeu-
systems such as nanoparticles), liposomes are very attractive and are the primary particulate carrier systems under investigation. Since the early 1970s, when Gregoriadis and Ryman proposed the use of liposomes as drug carrier systems, liposomes have been intensively studied for that application. Since their discovery by Bangham about 50 years ago, liposomes have become promising tools in drug delivery systems. This has increased the therapeutic index of many drugs, and offers improved drug targeting and controlled release.

Liposomes are composed of phospholipids self-assembled in concentric bilayers enclosing an aqueous space (Figure 1). The bilayer is impermeable to aqueous solutes, allowing hydrophilic drugs to be encapsulated in aqueous region of the liposomes, while hydrophobic compounds can be incorporated into the bilayer region.

For drug delivery, the liposomes are generally 50 to 150 nm in diameter. Larger liposomes are rapidly removed from the circulation. These lipid vesicles are unique in their ability to take drugs, but differ in physical and chemical properties such as polarity, charge and size.

Drugs in liposomes can be located either in lipid bilayers (with hydrophobic hydrocarbon chain located in the core) or at their high polar surface (which may be neutral or charged) and in the core of the liposomes.

Liposomes offer several advantages over other delivery systems, including biocompatibility, low immunogenicity, low toxicity and a wide range of physical properties that can be modified to control their biological activities.

<table>
<thead>
<tr>
<th>Phospholipid (R₁)</th>
<th>Hydrophobic chains (R₂, R₃)</th>
<th>Lipid Name (Abbreviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine CH₂CH₂N⁺(CH₃)</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₇C(O)⁻ (oleyl)</td>
<td>Dioleoylphosphatidylcholine (DOPC)</td>
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<tr>
<td></td>
<td>CH₃(CH₂)₁₂C(O)⁻ (myristoyl)</td>
<td>Dimyristoylphosphatidylcholine (DMPC)</td>
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<tr>
<td></td>
<td>CH₃(CH₂)₁₄C(O)⁻ (palmitoyl)</td>
<td>Dipalmitoylphosphatidylcholine (DPPC)</td>
</tr>
<tr>
<td></td>
<td>CH₃(CH₂)₁₆C(O)⁻ (stearoyl)</td>
<td>Distearoylphosphatidylcholine (DSPC)</td>
</tr>
<tr>
<td>Phosphatidylethanolamine CH₃CH₂NH₃⁺</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₇C(O)⁻ (oleyl)</td>
<td>Dioleoylphosphatidylethanolamine (DOPE)</td>
</tr>
<tr>
<td></td>
<td>CH₃(CH₂)₁₂C(O)⁻ (myristoyl)</td>
<td>Distearylphosphatidylethanolamine (DSPE)</td>
</tr>
<tr>
<td>Phosphatidylglycerol CH₃CHOHCH₂OH</td>
<td>CH₃(CH₂)₁₂C(O)⁻ (myristoyl)</td>
<td>Dimyristoylphosphatidylglycerol (DMPG)</td>
</tr>
<tr>
<td></td>
<td>CH₃(CH₂)₁₄C(O)⁻ (palmitoyl)</td>
<td>Dipalmitoylphosphatidylglycerol (DPPG)</td>
</tr>
<tr>
<td>Phosphatidylserine CH₃CHNH₂⁺COO⁻</td>
<td>CH₃(CH₂)₁₄C(O)⁻ (palmitoyl)</td>
<td>Dipalmitoylphosphatidylserine (DPPS)</td>
</tr>
<tr>
<td></td>
<td>CH₃(CH₂)₁₆C(O)⁻ (stearoyl)</td>
<td>Distearylphosphatidylserine (DSPS)</td>
</tr>
</tbody>
</table>
2. CLASSIFICATION OF LIPOSOMES

Conventional liposomes can possess different lipid compositions. Despite these favourable characteristics, the use of conventional liposomes as drug delivery systems in systemic, in vivo application has been seriously limited by their rapid clearance from the circulation resulting from their uptake into mononuclear phagocyte system (MPS). However, the natural affinity of macrophages for liposomes could be exploited for macrophage activation in the immunotherapy of cancer. Uptake by macrophages and changes in pharmacokinetic properties of liposomes are caused by their interaction with several plasma proteins adsorbing to the liposome surfaces. Several approaches to reduce this uptake by modifying the physical properties of liposomes have not significantly prolonged their half-life in the systemic circulation.

An important breakthrough in the stabilisation of liposomes and their prolonged systemic circulation occurred in the early 1990s, when the concept of steric stabilisation of liposomes was introduced. “Sterically stabilised liposomes” or long-circulating liposomes contain lipid components with bulky and highly flexible hydrophilic moieties, which reduce the unwanted interaction of liposomes with serum proteins. It was shown that incorporation in the bilayer membrane of polyethylene glycol (PEG) lipid derivatives significantly prolonged the circulation half-life of liposomes. Introduction of 5-10% of PEG lipid-derivatives build a fixed aqueous layer on the liposome surface, which shields surface charges, increases surface hydrophilicity, enhances repulsive interactions between polymer coated liposomes and blood components and forms a polymeric layer which is impermeable for large molecules. This discovery was significant in liposome research, which provided a safe synthetic carrier system that can be easily produced.

Conventional and long-circulating liposomes present a slow release of the drug. Therefore, polymorphic liposomes have been developed. These liposomes become reactive when pH, temperature or surface charge of the membrane change (pH-sensitive, thermo-sensitive and cationic liposomes).

The development of pH-sensitive liposome was proposed after the observation that some pathological tissues, including tumors or areas of inflammation and infection, as compared to normal tissues, reveal an acidic environment. A pH-sensitive liposome is generally stable at physiological pH but destabilize under acidic conditions, thus leading to the release of drugs encapsulated in aqueous core. The pH-sensitive liposomes consist mainly of phosphatidylethanolamine (PE) or its derivatives combined with amphiphilic compounds containing an acid group (e.g. carboxylic group) that acts as a stabilizer of the bilayer at neutral pH.

In order to further improve the specificity of liposomes for injured organs or tissues, targeted liposomal formulations with functionalised surface have been developed which represent the next step of liposomal drug delivery in medical treatment. These liposomes have specific ligands attached to the surface of liposomes. Ligands are substances with a high affinity for receptors or other substances expressed by injured cells or tissues. Ligands are capable of directing the liposomes to the region of interest in a process called active targeting. The ligand can be attached by covalent binding to the liposome surface or by electrostatic and hydrophobic insertion into the liposomal membrane.

3. IMMUNOLIPOSOMES

Due to the high specificity for their target antigens, monoclonal antibodies (mAbs) or their derivatives are often used as the ligands in the targeted liposomal formulations. Ligand targeted liposomes modified with mAbs or their derivatives are defined as immunoliposomes, which allows an active tissue targeting through binding to tumor cell-specific receptors. Tumor cells are often characterized by a specific expression pattern of membrane associated proteins. Cancer therapies that exploit targeted liposomal formulations to deliver attached cytotoxic drugs selectively to malignant cells are currently receiving significant attention and are being recognized as an effective strategy for increasing the therapeutic indices of anticancer drugs. Antibody modified liposomes attract great interest for their potential use in specific drug delivery to cancer cells, gene therapy, drug delivery through blood brain barrier or molecular imaging. Thus far, immunoliposomes show promising results in vitro and in vivo and ap-
IMMUNOLIPOSOMES – PROPERTIES AND IN-VIVO TRANSPORT

4. IMMUNOLIPOSOMES IN-VIVO

The critical parameter for targeting immunoliposomes to tumor is accessibility of tumor cells. Many tumors are located in places that are less accessible if liposomes are injected intravenously.

The process of the targeted drug delivery with immunoliposomes can be roughly divided into two phases:

– transport phase, in which immunoliposomes travel from place of administration (often intravenously) to the target cells and
– activity phase that includes the specific binding of immunoliposomes to target cells and subsequently delivery of incorporated drug.

There are the physiological and anatomical barriers that immunoliposomes encounter when administered intravenously. Although much attention has been focused on optimizing transport process of immunoliposomes, less work is done to improve effective drug delivery of immunoliposomes within tumor. Ideally, immunoliposomes should not release their drug contents during the transport phase, but to deliver effectively the encapsulated drug inside target cells.

4.1 Barriers in transport phase

Directly after intravenous administration, immunoliposomes were exposed to a number of factors that can compromise structural integrity of the

**Table 2. Components of immunoliposome design**

<table>
<thead>
<tr>
<th>Component</th>
<th>Consideration for optimal design</th>
</tr>
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| Target antigen | Homogeneously overexpressed  
Vital to tumor progression  
Does not solubilize into circulation |
| Antibody     | Humanized or human mAb fragment  
Efficiently endocytosed  
Intrinsic antitumor activity |
| Linkage      | Ab covalently attached to hydrophobic anchor  
Specific sites on Ab and liposome; avoids steric hindrance |
| Liposome     | Stable as intact construct in vivo  
Long-circulating  
Selective extravasation in tumors; small diameter to improve tumor penetration |
| Drug         | Efficient and high capacity encapsulation  
Increased efficacy with bystander effect  
Anticancer effect particularly suited to target cell population  
Cytotoxicity enhanced by binding of mAb |
liposomes. For example, after injection in the blood, the adsorption of serum proteins on the liposome surface can cause early release of incorporated drugs or aggregation of liposomes. Aggregates of liposomes will be quickly removed from circulation system by phagocytes of the liver and spleen. In addition, it was demonstrated that naturally occurring antibodies (IgM and IgG) showed reactivity with the major phospholipid groups present in the serum of rabbit and humans. The natural presence of polyclonal antibodies is able to activate complement via the alternative pathway and that way participate in complement-mediated lysis of liposomes.

4.1.1 Clearance of immunoliposomes

One of the most important barriers that limits application of immunoliposomes for targeted drug delivery is identification and removal from the blood of immunoliposomes by the cells of the mononuclear phagocyte system (MPS), particularly by macrophages in the liver and spleen. Additionally, the presence of the whole antibody that is conjugated to the surface of a liposome makes the immunoliposomes sensitive to the Fc receptors of phagocytes, which make immunoliposomes more prone to rapid clearance. The group of Fc receptors that express different MPS cells, connect the constant parts (Fc) of antibodies that results in an internalization of antibodies from opsonized complex (eg. bacteria). Similarly, immunoliposomes carrying the whole antibodies were quickly removed due to the exposed Fc parts.

Long-circulating liposomes with PEG chains attached to the surface show slow clearance from the circulation by mononuclear phagocyte system (MPS). Several methods for antibody attachment on the liposome surface have been developed.

Two main strategies are
a) attachment of the ligand into membrane bilayer and
b) coupling of the ligand with the PEG chain end (Figure 3).

It was shown that the rate of clearance of PEG immunoliposomes depends on the density of antibodies on the surface of liposomes. At low antibody density (< 50 mg mAb / mmol phospholipide), PEG immunoliposomes are cleared at a rate that is slightly faster than in PEG liposomes without antibody. At high antibody density (> 100 mg mAb / mmol phospholipide), clearance is very fast with a half-life of only a few minutes. Clearance is also associated with exposure of the Fc region of the antibody conjugated to the terminal ends of the PEG. A study in differences in the pharmacokinetics and biodistribution of PEG-immunoliposomes if Fab fragments were used instead of whole antibody showed that PEG-immunoliposomes with about 51 exposed whole antibody molecules conjugated at the terminal PEG chain ends are rapidly removed from circulation (60% after 1 hour), while the same type of PEG-immunoliposomes with attached approximately 517 Fab fragments derived from the same monoclonal antibodies showed six times longer circulation time.

Therefore, the use of Fab fragments rather than whole antibodies attached to the terminal ends of the PEG chain seems to have better properties to direct sterical stabilized liposomes to solid tumors.

4.1.2 Immunogenicity

Immunogenicity is an important factor which has to be taken into account in development of immunoliposomes. The presence of antibodies on the surface of liposomes can result in an immune response. This immunogenicity results in reduction of circulation time of immunoliposomes. Also, it was demonstrated that the antibodies...
that are attached to immunoliposomes are more immunogenic than antibodies in the free form\textsuperscript{15}. Increased immunogenicity is caused either by attachment of antibodies to immunoliposome or by chemical modification of antibodies that is needed for coupling reaction. Immunogenicity of antibodies may be decreased if smaller antibody fragments (such as Fab or scFv) or human antibodies are used.

4.1.3 The passage of immunoliposomes through the vascular endothelium

Immunoliposomes have to pass through the vasculature lining of the tumor in order to reach the tumor cells that are localized outside the blood. Normal vascular endothelium consists of a continuous lining of endothelial cells, which are very tightly associated with each other. Below this cell layer is the basement membrane and in the larger vessels there is also an additional layer of smooth muscle cells. This thick barrier prevents the passage of large particles and molecules. Blood vessels of solid tumors formed by the process of angiogenesis often show large shrinkage pores or fenestra (up to 400 nm) and irregularly formed basement membrane, which cause increased passage for large molecules\textsuperscript{16}. Therefore, at sites of tumors with increased vascular permeability, liposomes can break out of the vessel if they are small enough and have long circulation time. The enhanced permeability and retention effect describes the accumulation of liposomes in tumor tissues and subsequent release of active substances, due to fenestrations in the blood vessel’s endothelial layer and a significantly reduced lymphatic drainage in the tumor tissue\textsuperscript{17}.

4.1.4 Transport through the tumor

Tumors often develop in such way that it is very difficult for particles to penetrate intratumorally through the interstices\textsuperscript{18}. Fast-growing tumors often have poorly developed system of lymphatic drainage. This, together with increased vascular permeability results in increased interstitial pressure within the tumor, which hinders the transport of liposomes to the center of the tumor. In addition, although the process of angiogenesis may contribute to structural defects of the blood vessels which contribute to an increased permeability of those vessels, these defects are not distributed evenly within the tumor. As a result, the liposomes are heterogeneously distributed in the tumor and are predominantly located in the perivascular regions\textsuperscript{19}.

The binding site barrier hypothesis for antibodies is proposed, suggesting that immunoliposomes will not penetrate deep into the tumor, but rather bind to the first target cell that they encounter. In the case of solid tumors, the first target cells that intravenously applied immunoliposomes will encounter are those located directly behind the endothelial layer (perivascular zone). Furthermore, it was concluded that passive long-circulating liposomes show better penetration properties into solid tumors compared with immunoliposomes, therefore showing better anti-tumor activity\textsuperscript{20}. Additionally, whole antibodies may cause endocytosis of the Fc fragments by macrophages located in the perivascular area. Moreover, an expression of Fc fragments of activated endothelial cells is observed\textsuperscript{21}, which may also contribute to retention of perivascular immunoliposomes containing the whole antibody.

4.1.5 Binding to the tumor cell

Selection of target antigen - The molecular target of specifically directed therapies is another key factor for successful achievement of cytotoxic effects. The presence of a candidate molecular target on the surfaces of malignant or other cells or structures serves as a “gateway” to achieve the desired effect of cyto-reduction. While complete selectivity is practically impossible to achieve, several relevant criteria must be achieved to reach the highest level of selectivity. A high density and concentration of target antigen are very relevant\textsuperscript{22}. The target should trigger a distinct immune response to produce a good corresponding antibody. The domain of the antigen location must be accessible and thus on the cell surface. Accessible tumor-specific antigens or tumor-overexpressed antigens provide the most suitable targets. The higher overexpression level, the greater possibility for achieving selectivity.

A successful delivery of antineoplastic drugs by immunoliposomes implies that the drug is delivered to each tumor cell. Therefore, the liposomes should target the surface molecules located on
each single tumor cell and most important, these surface molecules should not be located on normal cells. Tumor cells are known for phenotype heterogeneity, also all malignant cells do not express identical target antigens. Targeting immunoliposomes on more than one surface antigen of tumor can reduce chance of positive selection of the population of tumor cells.

Steric stabilization and specific binding - delivery of immunoliposomes to target solid tumors requires leaking at the tumor site. For efficient leaking, liposomes with long circulation time are essential. The widespread method to achieve long circulation time is steric stabilization of the surface of liposomes with polyethylene glycol (PEG). Some studies showed that with an increase of the PEG density and size, the coupling efficiency of the antibody with the liposome bilayer decreases. Furthermore, interaction of antibody that is coupled on the bilayer of PEGylated liposomes, with its target antigen, can be hindered due to steric interference caused by long PEG chains. This steric hindrance problem is solved by coupling of antibodies or antibodies fragments to the terminal ends of PEG chains. These PEG-immunoliposomes combine possibilities of active targeting with long circulating properties.

4.1.6 Therapeutic availability

After binding to a tumor cell, encapsulated drug should become available for therapeutic effect. In general, the delivery of encapsulated drugs to tumor cells can occur by four different mechanisms:

1. Release of the encapsulated drug from the immunoliposomes surface and a subsequent intake of free drug by tumor cells;
2. Transfer of lipophilic substances from immunoliposomes bilayer to the plasma membrane of tumor cells;
3. Endocytosis of the surface receptors of immunoliposomes and release of encapsulated drugs within cells;
4. Fusion of immunoliposomes membrane with target cell membrane or endosomal membrane.

In the first mechanism incorporated drug is released extracellular, while other three mechanisms release the drug at or within a cell. In general, tumor cells circulating in the bloodstream require intracellular delivery because extracellular delivery results in rapid diffusion and redistribution of drugs in blood vessels. In the case of solid tumors, extracellular release seems better option because the drug diffuse within the tumor mass, allowing the drug to reach tumor cells that do not express target antigens or to those cells that are unattainable for relatively large immunoliposomes.

4.1.7 Selective transfer of lipophilic pro-drugs from immunoliposomes to tumor cells

Immunoliposomes are attractive system for delivery of lipophilic anticancer drugs or prodrugs to tumor cells. Lipophilic drugs or lipophilic derivatives (prodrugs) of hydrophilic drugs that are not effectively stored in the aqueous phase of the liposome can be incorporated in the bilayer of liposomes. Examples for those drugs are arabinofuranosilcitozin (Ara-C), 5-fluorouracil (5-FU), 5-fluoro-2-deoxyuridine (FUDR) and methotrexate. Immunoliposomes can mediate efficient intracellular delivery of a lipophilic drug without being internalized.

Some proteins which bind to a cell surface receptor are rapidly internalized by the cells, before they dissociate from their receptors. This process, called receptor-mediated endocytosis, is an important and general mechanism by which cells “take” nutritious and regulatory proteins from the extracellular fluid. This internalization process has demonstrated that small immunoliposomes targeted to specific surface receptors (epidermal growth factor receptor, transferrin receptor, folate receptor) are internalized in a way that is similar to the receptor-mediated endocytosis. Unfortunately, this process does not guarantee full availability of internalized therapeutic drug molecules.

Immunoliposomes that are internalized by mechanism of receptor-mediated endocytosis will finally end up in lysosomes where they will be digested along with the encapsulated drug molecules. Physico-chemical nature of the encapsulated drug can be adjusted to “escape” endosomal or lysosomal space. Molecules such as doxorubicin may be resistant to the activity of the enzyme.
lysozyme and also low pH and may be able to cross endosomal or lysosomal membrane. Also, lipophilic drugs can be transmitted during endocytosis from liposome on the endosome membrane. Endosomal escape of hydrophilic drug into the cytosol can be improved by incorporating the pH-dependent “fusogenic” substances within liposome.

Fusion of immunoliposomes with cells is an attractive strategy for the delivery of retained drug molecules inside the cytosol. In general, there are two principles that cytosolic delivery via fusion can be achieved:

1. fusion of immunoliposomes with plasma membrane of target cells that begins after immunoliposomes bind to target cells;
2. pH-dependent fusion of immunoliposomes with the endosomal membrane, after internalized with target cells through receptor-mediated endocytosis.

Fusion of immunoliposomes with plasma membrane of the target cells does not require internalization of immunoliposomes. Targeting of internalized epitopes is not needed, which increases the number of potential target epitopes for immunoliposomes. Fusion of immunoliposomes with the plasma membrane results in release of the encapsulated drug directly into the cytosol. However, as a result of nonspecific binding, immunoliposomes with fusogenic activity at neutral pH may be fused with the non-target cells.

Fusion with endosomal or lysosomal membrane requires internalization. For this purpose, immunoliposomes should target the receptors with known characteristics of internalization. In addition, the size of liposomes should not prevent the endocytosis and therefore should be preferably < 100 nm. Membrane active peptides can also be used to improve the cytosolic delivery of liposome-encapsulated drugs from endosomal- or lysosomal space. Such peptides may act by destabilizing membrane, formation of pores or by inducing membrane fusion.

5. CONCLUSION

Since their discovery about 50 years ago, liposomes have become promising tools in drug delivery systems. Development of a liposome and liposomal drug delivery system has led to an improvement effect of therapy using the new generation of drugs. This has increased the therapeutic index of many drugs, and offers improved drug targeting and controlled release.

There are several groups of liposomes: conventional liposomes (first generation, which were very unstable), stealth liposomes (whose modification provide increased stability), stimuli-sensitive liposomes, cationic liposomes (facilitating the transfer of DNA into living mammalian cells) and target liposomes (indentify target molecules through specific molecules or have linked target molecules such as antibodies, carbohydrates, peptides, growth factors on their surface). Antibodies and antibody fragments are the most widely used targeting moieties for liposomes due to the high specificity for their target antigens.

This has given rise to a new class of drug delivery vehicles, immunoliposomes. Immunoliposomes are very attractive drug-targeting systems for chemotherapeutic cancer treatment. One effective means of targeting tumors is via coupling of antitumor antibodies or portions of antibodies to immunoliposomes, which allows an active tissue targeting through binding to tumor cell-specific receptors. In cancer therapy, many factors must be taken into consideration, including adequate choice of target antigen, antibody function and antibody-liposome linkage. Thus far, immunoliposomes show promising results in vitro and in vivo and appear to be effective systems for improvements in cancer treatment.

In conclusion, immunoliposomes have shown a promising future as a new generation of cancer therapeutics. Certain critical questions and many obstacles still remain and more research is needed in field of immunoliposomes. However, more clinical data becomes available, further understanding will certainly lead to a more rational design of optimized immunoliposomes with improved selectivity, efficacy, and safety in cancer treatment. Additionaly, the future of drug therapeutics may not lie in the development of new chemical entities, but the modification of existing drug molecules. Such molecules using suitable carriers could be less toxic and have enhanced delivery to specific side and activity.
6. REFERENCES


