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Chapter 4

Liposomes as Carriers of Anticancer Drugs

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1. Introduction

Nanotechnology and nanoscience present a highly positive prospective of bringing benefits to many research areas and applications. Nanosized vehicles have received considerable attention over the past 30 years as pharmaceutical carriers with a wide range of applications, including drug delivery vehicles, adjuvants in vaccinations, signal enhancers/carriers in medical diagnostics and analytical biochemistry, solubilizers for various materials, as well as their role as a support matrix for chemical ingredients and as penetration enhancers in cosmetic products. More recent developments have reported on the field of liposomal drugs, from the viewpoint of clinically approved products, with cancer therapy representing the main area of interest [1-3]. In this context, liposomes can be used to improve current cancer treatment regimens due to their capacity to increase the solubility of poorly water-soluble antitumor drugs. Moreover, these also act to decrease the mononuclear phagocyte system’s (MPS) uptake by using long-circulating liposomes which promote a passive directing toward the tumor region and can lead to an active directing toward the tumor site by connecting specific ligands to the liposome surface [4,5]. These strategies minimize drug degradation and inactivation upon administration, as well as increase the drug’s bioavailability and the fraction of drug delivered within the pathological area, thus improving efficacy and/or minimizing drug toxicity.
2. Definition, structure, and classification of liposomes

Liposomes are spherical vesicles composed of one or more lipid bilayers, involving an aqueous compartment (Figure 1). These are formed spontaneously when the lipids are dispersed in an aqueous medium by stirring, in turn giving rise to a population of vesicles which may reach a size range from dozens of nanometers to dozens of microns in diameter [6]. The lipid molecules possess head groups which are attracted to water molecules and organize themselves in such a way as to point toward the aqueous cavity, whereas the hydrocarbon tails are repelled by the water molecules and point in the opposite direction.

The head groups of the inner layer point in the direction of the intravesicular fluid, with the tails pointing away from it. As such, the hydrocarbon tails of one layer point toward the hydrocarbon tails of the outer layer, in turn forming the normal bilipid membrane [3]. Once the liposomes have reached both the aqueous and lipid phases, they can encapsulate drugs with widely varying lipophilicities in the lipid bilayer, in the entrapped aqueous volume, or at the bilayer interface [7,8].

Biodegradable and biocompatible phospholipids and sphingolipids are the lipids that are most commonly used to prepare liposomes (Table 1 and Figure 2). These structural lipids can be of
either natural or synthetic origin, given that those of natural origin consist of a mixture of various lipids. In general, cylindrical molecular-shape lipids, such as phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, and sphingomyelin, are chosen for liposome formulations, as they organize into stable bilayers in aqueous solutions. Among these lipids, phosphatidylcholines are the most widely used due to their appropriate stability and their ability to act against changes in pH or salt concentrations in the product or/and biological environment [10].

Table 1. Examples of phospholipids used in liposome preparation.

<table>
<thead>
<tr>
<th>Phospholipid (R₁)</th>
<th>Hydrophobic chains (R₂,R₃) (name)</th>
<th>Lipid Name (Abbreviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine CH₂CH₂N⁺(CH₃)₃</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₇C(O)- (oleyl)</td>
<td>Dioleoylphosphatidylcholine (DOPC)</td>
</tr>
<tr>
<td></td>
<td>CH₃(CH₂)₁₄C(O)- (myristoyl)</td>
<td>Dimyristoylphosphatidylcholine (DMPC)</td>
</tr>
<tr>
<td></td>
<td>CH₃(CH₂)₁₂C(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 CH₂CH₂NH⁺</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₇C(O)- (oleyl)</td>
<td>Dioleoylphosphatidylethanolamine (DOPE)</td>
</tr>
<tr>
<td></td>
<td>CH₃(CH₂)₁₄C(O)- (stearoyl)</td>
<td>Distearoylphosphatidylethanolamine (DSPE)</td>
</tr>
<tr>
<td>Phosphatidylglycerol CH₂CHOHCH₂OH</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 CH₂CHNH⁺ ‘COO⁻’</td>
<td>CH₃(CH₂)₁₂C(O)- (palmitoyl)</td>
<td>Dipalmitoylphosphatidylserine (DPPS)</td>
</tr>
<tr>
<td></td>
<td>CH₃(CH₂)₁₆C(O)- (stearoyl)</td>
<td>Distearoylphosphatidylserine (DSPS)</td>
</tr>
</tbody>
</table>

Liposomes are mainly classified in terms of size (small, intermediate, or large), number of bilayers (uni- and multi-lamellar), composition and mechanism of drug delivery. Small unilamellar vesicles (SUV) consist of a single lipid bilayer with an average diameter ranging
from 25 to 100 nm. Large unilamellar vesicles (LUV) also consist of one lipid bilayer and are greater than 100 nm, whereas multilamellar vesicles (MLV) are made up of several concentric lipid bilayers and measure of 1-5 µm [7,11] (Figure 3). As regards the composition and mechanism of drug delivery, the liposomes can be classified as conventional liposomes, long-circulating liposomes, polymorphic liposomes (pH-sensitive, thermo-sensitive, and cationic liposomes), and decorated liposomes (surface-modified liposomes and immunoliposomes) (Figure 4).

Conventional liposomes can possess different lipid compositions; however, the most commonly used lipids are phosphatidylcholines and cholesterol (CHOL). A major drawback of conventional liposomes is their rapid uptake by MPS after systemic administration [8]. In the 1980s, the development of long-circulating liposomes boosted interest in the clinical application of liposomes as a drug delivery system for cancer treatment. Prior studies have shown that the presence of a dense glycocalyx with a high sialic acid content, used to produce a hydrophilic layer around the erythrocytes, prevented their destruction by MPS macrophages [12]. Allen and Chonn [13] applied this same concept to liposome development, incorpo-
rating purified glycolipids in the membranes of liposomes and testing their stability in mice. The results showed that the incorporation of monosialoganglioside GM1 and sphingomyelin acted synergistically to diminish the rate and extent of uptake of liposomes by macrophages in vivo. However, monosialoganglioside GM1 did present some inconveniences, such as the expensive extraction process and the brain, as a prime source, which was considered unsuitable for use in pharmaceutical products. Klibanov and coworkers [14] were the first to show that the incorporation in the bilayer membrane of polyethylene glycol (PEG) lipid derivatives, significantly prolonged the circulation half-life of liposomes. It could be observed that the introduction of five to ten percent of PEG lipid-derivatives prevents opsonization through the induction of 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 opsonin molecules even at relatively low polymer concentrations [15-18].

Figure 3. Classification of liposomes according to average diameter and number of bilayers.
discovery was a major breakthrough in liposome field research, supplying a safe synthetic compound that can be easily produced in mass scale.

Regardless of the strategies mentioned above, conventional and long-circulating liposomes may present a slow release of the active substance or may be unable to fuse with the endosome after internalization. As such, polymorphic liposomes have been developed to overcome these
problems, mainly due to the fact that these liposomes become reactive when submitted to membrane changes triggered by pH, variations in temperature, or surface charge alterations.

A pH-sensitive liposome is generally stable at physiological pH but can undergo destabilization and acquire fusogenic properties under acidic conditions, thus leading to the release of its aqueous contents [19,20]. The development of this kind of 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 [21]. The endosome formed during the cellular internalization of liposomes also presents an acidic pH. 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 (Figure 5). The PE presents a conic geometry, since it contains a less bulky polar group, as compared to its hydrocarbon chain. This fact allows for strong intermolecular interaction between amine and phosphate groups in the polar moiety of PE. The molecules organize in a structure, called the inverted hexagonal phase, in which the polar head of the phospholipid points toward the inner cavity, while the carbon chains point toward the outer areas. The introduction of carboxylated compounds among phospholipid molecules promotes the repulsion of the phosphate groups with the carboxylate groups, which is deprotonated at neutral pH, favoring the formation of the bilayer (lamellar phase). The exposure of pH-sensitive liposomes to acidic pH leads to the protonation of carboxylate groups, removing the repulsion with phosphates, in turn destabilizing the bilayer and releasing the encapsulated substances [19, 22]. Hong and coworkers [23] showed that pH-sensitive liposomes made up of DOPE/distearoylphosphatidylglycerol (DSPG)/distearoylphosphatidylethanolaminepolyethylene glycol2000 (DSPE-PEG2000), as compared to non-pH-sensitive liposomes made up of DPPC/CHOL/DSPE-PEG2000 are stable in plasma and are able to release an entrapped marker more rapidly within tumor tissues.

Lipid molecules are able to organize at the lamellar phase, depending on the temperature, molecular shape of the lipids, and the conditions in the lipid-water mixture (concentration and ionic strength). Lamellar phases are classified in crystalline lamellar ($L_c$), lamellar gel ($L_g$), and lamellar liquid-crystalline ($L_n$). Lipid phase-transitions occur at certain temperatures according to the conditions of the medium. The main phase transition occurs at the temperature in which the lipid membrane passes from a tightly ordered gel ($L_g$) to a fluid lamellar ($L_n$), where the freedom of movement of individual molecules is high.

Thermo-sensitive liposomes, another kind of polymorphic liposome, are vesicles that present a bilayer composition in which the phase-transition temperature is slightly above 37°C, as can be seen in DPPC or lipids attached to thermosensitive copolymers (N-isopropylacrylamide and N-acryloylpyrrolidine). The local release of drugs entrapped in these liposomes is triggered by hyperthermia. Cationic liposomes present a positive surface charge, due to the presence of cationic lipids; can fuse with cell or endosome membranes; and are suitable for the delivery of negatively charged macromolecules (DNA, RNA, and oligonucleotides) [10].

In an attempt to improve the specificity of liposomes for injured organs or tissues and to prevent their uptake by the healthy tissues, liposomes with a functionalized surface, called “decorated” liposomes, have been developed by binding specific ligands. These ligands are
substances with a high affinity for receptors or other substances overexpressed by injured cells or tissues. These are also either absent or minimally present in healthy tissues [24] and are capable of directing the liposomes to the region of interest in a process called active targeting. The ligand can be introduced by covalent binding to the liposome surface or by electrostatic and hydrophobic insertion into the liposomal membrane [10]. Some examples of ligands are listed in Table 2.

Figure 5. Main constituents of pH-sensitive liposomes and their structural representation (A) - DOPE and cholesteryl hemisuccinate (CHEMS). DOPE molecules alone will form the inverted hexagonal phase (B, upper). The introduction of CHEMS allows for the formation of the lamellar phase, which corresponds to the formation of liposomes (B, mid). When in contact with acidic pH, the liposomes undergo destabilization and return to the inverted hexagonal phase (B, low).
<table>
<thead>
<tr>
<th>Ligand</th>
<th>Target</th>
<th>Some types of cancer that overexpress the target (cell lines)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb 2C5</td>
<td>Surface-bound nucleosomes</td>
<td>Brain cancer (U-87)</td>
<td>[25]</td>
</tr>
<tr>
<td>mAb C225 (Cetuximab)</td>
<td>EGFR</td>
<td>Several types of tumor</td>
<td>[26]</td>
</tr>
<tr>
<td>scFv C10 (derived from mAb anti-human EGFR)</td>
<td>EGFR</td>
<td>Several types of tumor</td>
<td>[26]</td>
</tr>
<tr>
<td>mAb αCD19</td>
<td>CD19</td>
<td>Lymphomas and leukemias (B Cells)</td>
<td>[27]</td>
</tr>
<tr>
<td>mAb αCD20 (rituximab)</td>
<td>CD20</td>
<td>Lymphomas and leukemias (B Cells)</td>
<td>[27]</td>
</tr>
<tr>
<td>rhu-mAbHER2-Fab (Fab’ of trastuzumab) (derived from mAb anti-human HER2)</td>
<td>HER2</td>
<td>Some types of breast cancer (BT-474 or MCF-7)</td>
<td>[28]</td>
</tr>
<tr>
<td>scFv F5 (derived from mAb anti-human HER2)</td>
<td>HER2</td>
<td>Some types of breast cancer (BT-474 or MCF-7)</td>
<td>[28]</td>
</tr>
<tr>
<td>Fab 222-1D8 (Fab’ of mAb anti-human MT1-MMP)</td>
<td>MT1-MMP</td>
<td>Several types of tumor</td>
<td>[29]</td>
</tr>
<tr>
<td>anti-TfR scFv</td>
<td>TfR</td>
<td>Several types of tumor</td>
<td>[30]</td>
</tr>
</tbody>
</table>

**Surface modified liposomes with small molecules or peptides**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Target</th>
<th>Some types of cancer that overexpress the target (cell lines)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGD</td>
<td>Integrins</td>
<td>Melanoma (A375 and B16)</td>
<td>[31]</td>
</tr>
<tr>
<td>Transferrin</td>
<td>TfR</td>
<td>Several types of tumor</td>
<td>[32]</td>
</tr>
<tr>
<td>Estrone</td>
<td>ER</td>
<td>Some types of breast cancer (BT-474 or MCF-7)</td>
<td>[33]</td>
</tr>
<tr>
<td>Folate</td>
<td>FR</td>
<td>Ovarian carcinoma (KB)</td>
<td>[34]</td>
</tr>
</tbody>
</table>

*mAb = monoclonal antibody; scFv = single chain variable fragment; RGD = Arginine-Glycine-Aspartic acid peptide; EGFR = Epidermal growth factor receptor; CD 19 = B-lymphocyte antigen CD19; CD 20 = B-lymphocyte antigen CD20; HER2 = Human epidermal growth factor receptor 2; MT1-MMP = membrane type-1 matrix metalloproteinase; TfR = Transferrin receptor; ER = estrogen receptor; FR = Folate receptor.*
3. Methods of liposome preparation

As aforementioned, liposomes are spontaneously formed when phospholipids are hydrated. Additional steps are often necessary to modify the size distribution and lamellarity of liposomes. Liposome preparation involves three major steps: vesicle formation, vesicle size reduction, and purification. Several preparation methods have been established based on the scale of the production and other considerations, such as drug encapsulation efficiency, the drug’s physicochemical characteristics, and the administration route (Table 3).

<table>
<thead>
<tr>
<th>VESICLES FORMATION</th>
<th>LIPOSOMES’ TYPES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid hydration followed by vortex or manual stirring</td>
<td>MLV</td>
</tr>
<tr>
<td>Reverse-phase evaporation</td>
<td>MLV, LUV</td>
</tr>
<tr>
<td>Organic solvent injection</td>
<td>MLV, LUV, SUV</td>
</tr>
<tr>
<td>Freeze-thawing</td>
<td>MLV, LUV</td>
</tr>
<tr>
<td>pH gradient</td>
<td>LUV, SUV</td>
</tr>
<tr>
<td>Dehydration-rehydration</td>
<td>MLV</td>
</tr>
<tr>
<td>Detergent dialysis</td>
<td>MLV, LUV</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VESICLE SIZE REDUCTION</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrusion through polycarbonate membranes</td>
<td>LUV, SUV</td>
</tr>
<tr>
<td>High-pressure homogenization</td>
<td>LUV, SUV</td>
</tr>
<tr>
<td>Microfluidization</td>
<td>Mainly SUV</td>
</tr>
<tr>
<td>Sonication</td>
<td>Mainly SUV</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PURIFICATION</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation</td>
<td>-</td>
</tr>
<tr>
<td>Dialysis</td>
<td>-</td>
</tr>
<tr>
<td>Column chromatography separation</td>
<td>-</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. Methods of liposomes preparation. For more details see [6, 11, 35].

The most commonly used methods for liposome preparation are lipid hydration and the replacement of organic solvents by an aqueous media (reverse-phase evaporation and organic-solvent injection). The lipid hydration followed by vortex or manual stirring, also known as Bangham’s method, consists of dissolving the lipids in a suitable organic solvent, such as chloroform or methanol. This process is then followed by removing the solvent under reduced pressure, by rotary evaporation, until a thin film has been formed. After, the thin film is hydrated in an aqueous medium, above the phase-transition temperature, resulting in the
formation of MLV liposomes (Figure 6). This is the simplest method of vesicle formation; however, it is limited in use due to its low encapsulation ability [36,37].

Figure 6. Representation of liposome production by lipid hydration followed by vortex or manual stirring.

All methods based on the replacement of an organic solvent by an aqueous media show that the solvents, whether miscible or immiscible with water, are replaced by an aqueous solution. First, the water-immiscible organic solution containing lipids is injected into the aqueous phase (reverse-phase method), or the stepwise addition of the organic phase (specifically, ethanol) is injected into the aqueous phase (organic solvent injection method), followed by the removal of the solvent. These methods are able to form liposomes with a high encapsulation percentage of both hydrophilic and lipophilic substances. Generally, the incorporation of lipophilic drugs is performed through their codissolution with the lipids [37]. Hydrophilic drugs are dissolved in the aqueous medium, whereas amphiphilic drugs can be dissolved in both mediums. The processes of liposome preparation can result in the formation of large vesicles (MLV) with heterogeneous size distribution; therefore, it is important to calibrate the formulation using a vesicle size reduction method (Table 3).

4. Liposome characterization

The behavior of liposomes in storage conditions and biological mediums is determined by specific factors, such as the size and surface charge of vesicles, chemical composition, membrane permeability, quantity of entrapped solutes, as well as the quality and purity of raw materials. Thus, it is of utmost importance to have as much information as possible regarding these parameters [6].
Bilayer constituents are responsible for the shelf-life; interactions with biological components, such as specific tissues, cells, and proteins; as well as the kinetics of the release of the entrapped drug in liposomes. The size of the liposomes influences their in vivo distribution, as this factor can determine the amount of time that the liposomes will remain in the bloodstream before being removed. By contrast, the surface charge of vesicles influences their physical stability due to the possible occurrence of fusion and/or aggregation phenomena [6]. Therefore, detailed chemical, physical, and physicochemical characterizations are important in an attempt to ensure the efficacy and stabilization of the liposome formulation.

Chemical analyses include the quantification of phospholipids and lysophospholipids, the evaluation of lipid oxidation, and the determination of the encapsulation percentage. As phospholipids represent the main constituents of the lipid bilayer, their quantification is important in evaluating the efficiency of the preparation method. Two degradation pathways have been described for phospholipids in aqueous liposomal dispersions: oxidative and hydrolytic degradation. The ester groups of the phospholipids can be hydrolyzed in the presence of water, producing lysophospholipids, a high concentration of which commonly leads to an increased permeability of the lipid bilayer and a destabilization of the system [38].

The oxidative pathway mainly involves phospholipids with unsaturated fatty acyl chains and tends to occur through the free radical mechanism. Lipid oxidation changes the bilayer’s integrity, commonly resulting in drug leakage, in turn inducing aggregation and/or fusion phenomena. Another important chemical characterization is the encapsulation percentage, which is the ratio between the amount of drug already contained within the liposomes and the amount of drug added to the liposome at the beginning of the preparation. In vivo efficacy of the liposomes, as well as their physical and physicochemical properties, depends on the total amount of drug encapsulated within the liposome.

Physical characterization consists of determining the size, surface charge, and lamellarity of the liposomes. As the performance of liposomes in vivo and physical stability strongly depend on the vesicle size, liposome size distribution should be determined during the preparation process and storage. On the other hand, the nature and density of the charge on the liposome surface are important parameters that influence the mechanism and extent of liposome-cell interaction. Furthermore, the retention of the superficial charge for long periods during storage contributes to the high physical stability of the formulation.

Concerning the physicochemical characterization, the main evaluated parameters include the lipid phase and the phase-transition temperature. The determination of phase transitions and the fluidity of the bilayer are important in the production and application of liposomes, since the behavior of the liposome membrane determines the permeability, fusion/aggregation, and protein binding, thus influencing the stability of liposomes and their kinetics in biological systems.

The methods most commonly used for liposome characterization, according to the parameters described above, are listed in Table 4.
<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>METHODOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids quantification</td>
<td>Lipid phosphorus content (Bartlett method)</td>
</tr>
<tr>
<td>Lysophospholipids quantification</td>
<td>Liquid chromatography combined with Bartlett method</td>
</tr>
<tr>
<td>Lipid oxidation</td>
<td>Spectroscopy, thin layer chromatography (TLC), high-performance liquid</td>
</tr>
<tr>
<td></td>
<td>chromatography (HPLC), gas-liquid chromatography (GC)</td>
</tr>
<tr>
<td>Determination of the encapsulation</td>
<td>Spectrophotometry, fluorescence spectroscopy, enzyme-based methods,</td>
</tr>
<tr>
<td>percentage</td>
<td>electrochemical techniques and HPLC</td>
</tr>
<tr>
<td>Size</td>
<td>Static and dynamic light scattering, microscopy techniques (light, electronic</td>
</tr>
<tr>
<td></td>
<td>and atomic force), size-exclusion chromatography, field-flow</td>
</tr>
<tr>
<td></td>
<td>fractionation and analytical centrifugation</td>
</tr>
<tr>
<td>Surface charge</td>
<td>Photon correlation spectroscopy associated with the electrophoretic</td>
</tr>
<tr>
<td></td>
<td>mobility</td>
</tr>
<tr>
<td>Lamellarity</td>
<td>Nuclear magnetic resonance (31P-NMR), electron microscopy, small angle</td>
</tr>
<tr>
<td></td>
<td>X-ray scattering</td>
</tr>
<tr>
<td>Lipid phase</td>
<td>X-ray diffraction, differential scanning calorimetry</td>
</tr>
<tr>
<td>Phase-transition temperature</td>
<td>Differential scanning calorimetry and nuclear magnetic resonance (31P-</td>
</tr>
<tr>
<td></td>
<td>NMR or 1H-NMR)</td>
</tr>
</tbody>
</table>

Table 4. Major methods of liposomes characterization. Based on [39, 40].

5. Strategies to optimize liposome stability: Focus on freeze-drying

As for any new high-tech product, the transfer from academic research to an industrial enterprise is crucial. Any commercial product involving a liposome formulation must contain well-defined stability characteristics and a shelf-life of more than one year. In this context, it is currently possible to obtain a reproducible preparation of large volumes of stable liposomes, and, in most cases, long-term stability problems have also been successfully solved [7].

The stability of liposomes is of major concern in their development for pharmaceutical applications. However, the potential application of liposomes as therapeutic tools is challenged by their inherent physical and chemical instability in aqueous mediums, which can result in an increased bilayer permeability and subsequent drug leakage, vesicle aggregation/fusion, and precipitation [41]. These instabilities can be stimulated by bilayer defects induced by chemical degradation (e.g. lipid oxidation and hydrolysis); by physical factors, such as heating or freezing; or due to phase transitions that occur when these aqueous dispersions are stored for extended periods [42,43].

The major approach to increase liposome stability is to establish an appropriate formulation, which requires the selection of the appropriate lipid composition and concentration, as well as the addition of other substances to improve its shelf-life. For example, the inclusion of cholesterol and its derivatives can reduce the permeability of the lipid bilayer. As unsaturated lipids commonly suffer peroxidation, the use of antioxidants and metal chelators may be necessary. Furthermore, it is of utmost importance to avoid the presence of oxygen both in the
form of dissolved oxygen and in the headspace of the container. Liposomes in an aqueous dispersion can also be hydrolyzed to form lysophospholipids and fatty acids. This process is catalyzed by hydroxyl and hydrogen ions and can be diminished by pH control, i.e., by adding a neutral buffer [44].

Beyond formulation optimization, many methods available for the stabilization of liposomes have been investigated, such as freeze-drying and spray-drying. Freeze-drying is the main approach used to extend the shelf-life of liposomes, especially for thermosensitive drugs encapsulated within liposomes [43].

Freeze drying, also known as lyophilization, is a complex drying process employed to convert solutions of labile materials into solids of sufficient stability for distribution and storage. Freeze-drying is an industrial process which consists of removing the water from a frozen sample by sublimation and desorption through a vacuum process. Nevertheless, this process generates a wide range of stress, including fusion and drug loss, during the freezing and drying steps when conducted without the proper stabilizers [42,45]. To promote the stability of the vesicles during freeze-drying, cryoprotectants, such as saccharides and their derivates (e.g. sucrose, trehalose, hydroxypropyl-β-cyclodextrin (HP–CD), are employed [46,47].

It is generally accepted that sugars can depress the main phase transition temperature ($T_m$) from the lamellar gel ($L_{\beta}$) to the lamellar liquid-crystalline ($L_{\alpha}$) phase during drying. Two main hypotheses were proposed to explain this depression effect of sugars: water replacement and vitrification.

Water replacement is the earliest established and the most widely accepted mechanism of membrane stabilization by sugars. It has been proposed that specific and particular interactions between phospholipids and sugars are required to produce the protective effect. Water is generally found around the polar head groups, with a slight penetration within the ester region between the glycerol backbone and the fatty acid residues. Accordingly, studies have shown that the interactions occur through the hydrogen bond between hydroxyl groups of the sugars and the phosphate groups on the bilayer surface. In summary, the sugars reduce the interactions between the water and phospholipids, and then the water is replaced [43,48]. It could be observed that trehalose, which has been considered an anomalous sugar in some studies, can also penetrate deeply into the membrane and form hydrogen bonds with the carbonyl groups of the phospholipids [49,50,51]. Therefore, trehalose seems to have a higher affinity for bonding with phospholipids.

The vitrification hypothesis is based on the effect of the hydration’s repulsive force, which separates the membrane phospholipids when there is an excess of water. During drying, when the water content, or the hydration repulse, is lowered, the compressive stress will increase. Vitrification states that sugars limit the close approach of phospholipids in the lamellar liquid-crystalline-to-lamellar gel phase transition through their nonspecific effects (no particular sugar-lipid interaction is required), namely, osmotic and volumetric properties as well as vitrification. The increase in the osmotic pressure of the solution, due to the presence of sugars, confines the water removal from the interface of the membranes. A high osmotic pressure leads to a low suction of any water molecules; therefore, less water is removed. Furthermore, the
molecular volume of moderately large sugars will maintain the phospholipid molecules separate. A further reduction of the stress levels occurs when the sugars do not crystallize, but rather vitrify in the membrane space during drying. It has been proposed that the rigidity or mechanical resistance of the glassy solid makes it more difficult for the membranes to reduce their spatial distance under compressive stress [52].

It should be noted that mechanisms of water replacement and vitrification are not mutually exclusive. The more important issue is the determining factor for \( T_m \) depression. According to the former hypothesis, it has been reported that vitrification is often required for the stabilization of the membrane but is not sufficient on its own [53]. Alternatively, it has also been proposed that specific sugar/lipid interaction may well exist but contributes little to the effect of preventing an increase in \( T_m \) without the vitrification of sugars [48].

In addition to stability, discussed above, other criteria must also be fulfilled to provide the acceptance of liposomes as pharmaceuticals. An efficient and adequate process for the preparation of sterile, pyrogen-free liposomes, by parenteral route, should be developed on an industrial scale. Furthermore, the final product must contain high and reproducible levels of drug entrapment, with minimal amounts of free drugs.


To develop pharmaceutical products, preclinical studies of pharmacodynamic, pharmacokinetic, and toxicological properties are required by regulatory agencies as part of procedures that must be followed prior to beginning clinical trials [54]. The Food and Drug Administration (FDA) requires that animal studies be reasonable predictors of the pharmacological activity of the investigated agent. In addition, toxicity studies should also be used to reveal adverse events that could be relevant to humans [55].

Pharmacodynamic studies include the characterization of action mechanisms, resistance, and treatment schedules, as well as the evaluation of the pharmacological activity in vivo. Although many drugs do act strongly against cancer, their use is commonly limited due to their toxic effects. Consequently, the definition of a toxicity profile is essential for the development of new drugs.

Concerning antitumor therapy, the primary role of preclinical toxicology is to identify a safe starting dose for Phase I trials, in addition to a potential for toxicity and its reversibility. The evaluation of toxicity includes pharmacological safety studies; single and repeated dose toxicity studies; as well as genotoxicity/carcinogenicity, reproductive toxicity, and local tolerance studies. Furthermore, wherever possible, pharmacokinetic/toxicokinetic studies should be included to define pharmacological endpoints related to both toxicity and efficacy for their use in the design of Phase I trials [54].

Liposomes have been used as carriers of platinum compounds (cisplatin and oxaplatin), anthracyclines (doxorubicin and daunorubicin), paclitaxel, camptothecin derivatives, antime-
tabolites (methotrexate, cytarabine), and Vinca alkaloids (vincristine, vinblastine and vinorelbine), aimed at reducing the toxic side-effects of cytostatic drugs without hampering their efficacy [56]. Their applications are based on the ability of liposomes to modify the tissue distribution of the entrapped drug, which becomes dependent on the physicochemical features of the liposomes and not the encapsulated content [57-59]. In addition, in cancer chemotherapy, the passive targeting of liposomes takes advantage of the inherent size of nanoparticles and the unique properties of tumor vasculature. As tumors grow and begin to outstrip the available supply of oxygen and nutrients, they release molecules that recruit new blood vessels to the tumor in a process called angiogenesis. Unlike the tight blood vessels in normal tissues, angiogenic blood vessels in tumor tissues contain gaps as large as 600 to 800 nm between adjacent endothelial cells. This dysregulated nature of tumor angiogenesis, coupled with poor lymphatic drainage, induces an enhanced permeability and a retention effect (EPR). Therefore, long-circulating liposomes will preferentially extravasate from these abnormal vessels and can selectively accumulate within the tumor interstitium [8,60-62].

6.1. Platinum compounds

Cisplatin (CDDP) (Figure 7) is one of the most effective chemotherapeutic agents used by intravenous route in the treatment of ovary, lung, testicle, head, and neck carcinomas [63-69]. Furthermore, CDDP has been widely used in the treatment of peritoneal carcinomatosis by intraperitoneal route. However, the administration of CDDP by both routes is still hindered by toxicity, mainly nephrotoxicity. Conventional liposomes composed of phosphatidylcholine/phosphatidylserine/CHOL containing CDDP were evaluated in IgM immunocytoma-bearing LOU/M rats. The results showed a lower incidence and severity of renal lesions after the liposomal formulation injection as compared to the free CDDP formulation. By contrast, the antitumor activity of this liposomal CDDP was similar to that of free CDDP, and the encapsulation of CDDP within this liposome formulation was unable to overcome drug resistance [70]. Newman and coworkers [71] developed a long-circulating formulation composed by hydrogenated soy phosphatidylcholine/DSPE-PEG_{2000}/CHOL (SPI-077) and performed in vivo studies using both C26 colon carcinoma and the Lewis lung tumor model. SPI-077 exhibited a 55-fold lower distribution volume and a 60-fold larger plasma area under the concentration–time curve (AUC). An increased tumor platinum uptake and a significantly improved antitumor effect could be observed with the use of SPI-077, as compared to free CDDP [72]. The experience from several clinical trials (phase I/II) with SPI-077® indicated a promising toxicity profile; however, the therapeutic efficacy might be hampered by an unsatisfactory release of CDDP from the liposomes. In a phase I study performed with 27 adult patients, no antitumor efficacy after SPI-077® treatment, along with relatively low levels of platinum-DNA adducts in tumor samples, could be observed [73].

Another long-circulating liposomal formulation containing CDDP made up of soy phosphatidylcholine (SPC)/ DPPG/CHOL/DSPE-PEG_{2000} is called Lipoplatin®. This formulation was developed to reduce the systemic toxicity of CDDP while simultaneously improving the targeting of the drug to the primary tumor and metastasis by enhancing the circulation time in body fluids and tissues [74]. Cytotoxicity studies of this formulation were performed in cell
lines derived from non-small cell lung cancer, renal cell carcinoma, and in normal hemato‐
poietic cell precursors. Lipoplatin®, when compared to CDDP, produced a stronger cytoxic
effect in both evaluated tumor cells lines and a lower toxicity in normal bone marrow stem
cells [75]. Fielder and coworkers [76] investigated whether the cytotoxic effect of Lipoplatin®
is dependent on the function integrity of DNA mismatch repair and concluded that this
function is a key determining factor accounting for the cytotoxicity of lipoplatin. Antitumor
efficacy of Lipoplatin® was assessed in xenografts of human breast, prostate, and pancreatic
cancer, where a reduction in tumor size could be observed. Histopathological analyses of the
tumors showed apoptosis in the tumor cells in a mechanism similar to that of CDDP [77].
Concerning toxicity, mice and rats treated with CDDP developed renal insufficiency with clear
evidence of tubular damage, but those treated with the same dose of Lipoplatin® were
completely free of kidney injury [78]. In addition, Lipoplatin® was safely administered to
normal dogs at doses of up to 150 mg/m² without the need for concurrent hydration protocols
[79]. As regards clinical trials, Stathopoulos and coworkers [74] investigated the pharmacoki‐
etics and toxicity of Lipoplatin® (25-125 mg/m²) in patients with pretreated advanced
malignant tumors. Measurement of platinum levels in the plasma of patients as a function of
time showed that a maximum platinum level is attained at 6-8 h. The half-life of Lipoplatin®
was 60-117 h, depending on the dose. Urine excretion reached approximately 40% of the
infused dose in 3 days. Grades 1 and 2 gastrointestinal tract and hematological toxicities were
detected after the administration of the highest dose. No nephrotoxicity could be observed.
Boulkas and coworkers [80] explored the hypothesis that intravenous infusion of Lipopla‐
atin® can result in preferential tumor uptake in clinical trials. The determining of platinum levels
in excised tumors and normal tissues showed that Lipoplatin® has the ability to preferentially
concentrate on the malignant tissue (10-50 fold) of both primary and metastatic origin, as
compared to adjacent normal tissue, following intravenous infusion in patients. Two phase I
and I-II studies were carried out to investigate the maximum tolerated dose (MTD) as well as
the dose-limiting toxicity (DLT). The first trial was conducted using a combination of Lipo‐
platin® and gemcitabine in patients with pretreated advanced pancreatic cancer, refractory to
prior chemotherapy with gemcitabine. The results showed an absence of nephrotoxicity after
administration of Lipoplatin® at doses of 100 and 125 mg/m². However, grade 2 neutropenia
and grade 1 nausea/vomiting, fatigue, diarrhea, neurotoxicity, and thrombotic episodes could
be observed after the administration of Lipoplatin® at similar doses. Thus, the DLT and MTD
to Lipoplatin, established in combination with 1000 mg/m² of gemcitabine, were 125 and 100
mg/m², respectively.
The combination achieved a partial response in 8.33% of the patients, disease stability in 58.3%, and clinical benefit in 33.3% [81]. In the second study, similar DLT and MTD were defined in patients with refractory or resistant non-small cell lung carcinoma [82]. However, as lipoplatin was combined with gemcitabine, the latter can be responsible for the toxicity observed. In this context, the administration of single Lipoplatin® was also tested and nephrotoxicity, gastrointestinal toxicity, and myelotoxicity were investigated as the main adverse reactions. From this study, DLT and MTD values were found for Lipoplatin® at 350 mg/m² and 300 mg/m², respectively. The dose of 350 mg/m² was not accompanied by nephrotoxicity, only by gastrointestinal side effects and grade 1-2 myelotoxicity. It seems that the dose of Lipoplatin® can reach a level that is double or even higher than that of CDDP without increasing toxicity [83]. A phase II study combining Lipoplatin® and vinorelbine in the first-line treatment of HER2/neu-negative metastatic breast cancer was also conducted [84]. The results showed complete response in 9.4% of the patients, partial response in 43.8%, stable disease in 37.5%, and progressive disease in 9.4%. In addition, this regimen was well tolerated and no grade 3/4 nephrotoxicity and neurotoxicity could be detected. In another phase II trial, Lipoplatin® (120 mg/m² given on days 1, 8, 15), administered in association with gemcitabine (1000 mg/m² given on days 1, 8) in inoperable (stage IIB/IV) non-small cell lung cancer, showed a better response rate (31.7%) than those treated with CDDP associated with gemcitabine (25.6%). Furthermore, lower nephrotoxicity after Lipoplatin® treatment, as compared to CDDP treatment, could be observed [85]. The first phase III clinical trial reported is a randomized, multicenter safety and efficacy study in patients with advanced squamous cell carcinoma of the head and neck. The pharmacokinetic profile of Lipoplatin® in combination with 5-fluorouracil showed that the liposomal formulation has a greater body clearance and a shorter half-life than does free CDDP, which confirms the clinical observation of decreased toxicity, especially nephrotoxicity [86]. The efficacy results showed 38.8% and 19% objective partial remission after treatment with free CDDP and lipoplatin, respectively. On the other hand, 64% of the patients achieved a stable disease after Lipoplatin® treatment, as compared to 50% of the patients that received CDDP [87]. In a second phase III trial, Lipoplatin® was much more well-tolerated than was CDDP in non-small cell lung cancer. Chemotherapy-naive patients received either 200 mg/m² of liposomal CDDP and 135 mg/m² paclitaxel (arm A) or 75 mg/m² of liposomal CDDP and 135 mg/m² of paclitaxel (arm B), once every 2 weeks. Arm A patients showed statistically significant lower nephrotoxicity, grade 3 and 4 leucopenia, grade 2 and 3 neuropathy, nausea, vomiting, and fatigue. There was no significant difference in the median and overall survival and in time to tumor progression (TTP) between the two arms; the median survival was 9 and 10 months in arms A and B, respectively, while TTP was 6.5 and 6 months in arms A and B, respectively [88]. Therefore, phase I, II, and III trials have shown that Lipoplatin® presents similar antitumor efficacy to CDDP in pancreatic, head and neck, breast cancers, and non-small cell lung carcinoma, as well as reduced toxicity, mainly nephrotoxicity. Preliminary studies have shown that Lipoplatin® is a candidate to be used in patients with renal failure [89].

Hirai and coworkers [68] encapsulated CDDP into liposomes and further conjugated the CDDP liposomes (CDDP-Lip) with a tetrasaccharide carbohydrate, Sialyl Lewis® (CDDP-SLX-Lip). These liposomes consisted of DPPC/CHOL/ganglioside/dicetylphosphate/dipalmitoylphosphatidylethanolamine (DPPE) at the molar ratio of 35:40:5:15:5, respectively. A549 tumor-
bearing mice treated with CDDP-SLX-Lip showed a survival rate of 75% at 14 days, even when a lethal level of CDDP was injected. Loss of body weight was negligible, and no histological abnormality could be found in many of the normal tissues. Accumulation of CDDP-SLX-Lip was approximately 6 times more than that of CDDP-Lip or CDDP. Therefore, a better antitumor activity could be observed for CDDP-SLX-Lip than for CDDP-Lip, with significantly less toxic effects in normal tissues.

Although CDDP is one of the most widely used chemotherapeutic agents, the development of tumor cell resistance against CDDP is a limitation in the clinical application of this drug. In this context, Krieger and coworkers [69] performed in vitro studies which demonstrated that liposomes have the potential to overcome the chemoresistance of tumor cells. The lipid composition of liposomes contained SPC/CHOL/distearoylphosphatidylethanolaminepolyethylene glycol (DSPE-PEG) in a 65/30/5 molar ratio, respectively. In these studies, PEGylated CDDP-containing liposomes were prepared, and the targetability of transferrin receptors (TfR) to correlate CDDP cell uptake with cytotoxicity in sensitive and CDDP resistant ovarian cancer cells (A2780), as compared to the free drug, was analyzed. Cytotoxicity proved to be even higher for liposomes, as compared to free CDDP, in the resistant cells after 24, 48, and 72 h, and slightly lower in the sensitive cells.

Júnior and coworkers [90] developed long-circulating and pH-sensitive liposomes containing CDDP (SpHL-CDDP), which were made up of DOPE/CHEMS/DSPE-PEG2000 at a molar ratio of 5.7:3.8:0.5, respectively. In an acid medium, such as tumor sites, CHEMS molecules undergo protonation, followed by the destabilization of liposomes and the release of CDDP. Thus, it is expected that the released CDDP in this specific site can improve the antitumor effect and reduce, or even eliminate, the side effects. Studies were carried out concerning the stability, cytotoxicity, and accumulation of this new formulation in a human small-cell lung carcinoma cell line (GLC4), as well as in its resistant subline. These liposomes were stable in plasma, circumvented the preclinical resistance to treatment with CDDP, and were able to introduce the same level of CDDP within resistant and sensitive cells. Biodistribution studies have demonstrated the ability of SpHL-CDDP, as compared to the injection of free CDDP, to promote a higher concentration and affinity of CDDP in Ehrlich solid tumors, as well as a lower renal perfusion of the anticancer agent after intravenous administration [90]. CDDP has also been widely used in the treatment of peritoneal carcinomatosis by the intraperitoneal (i.p.) route. However, CDDP, a low-molecular-weight compound, is rapidly absorbed by the capillaries in the i.p. serosa and transferred to the bloodstream, inducing the appearance of systemic side-effects, such as nephrotoxicity. Furthermore, i.p. CDDP chemotherapy is limited to patients whose residual tumor nodules are less than 0.5 cm in diameter after surgical debulking [91]. The failure of i.p. therapy is attributed to the poor penetration of CDDP within larger tumor masses. To achieve an optimal drug penetration within the tumor, the use of a high concentration and a longer time of contact with the tumor are required. In this context, Araújo and coworkers [92] evaluated the tissue distribution of SpHL-CDDP after their i.p. administration in Ehrlich ascitic tumor-bearing mice. The CDDP AUC obtained for ascitic fluid and blood after SpHL-CDDP administration was 3.3-fold larger and 1.3-fold lower, respec-
tively, when compared with free CDDP treatment, thus indicating its high retention within the peritoneal cavity.

In addition, MTD values obtained after i.v. and i.p. administration of SpHL-CDDP in healthy mice were approximately three times higher than those obtained using free CDDP. Hematological investigations revealed no alterations in red and white blood cell counts upon i.v. and i.p. administration of SpHL-CDDP at a dose corresponding to the MTD in mice. In addition, SpHL-CDDP treatment caused no pronounced alterations in the blood urea and creatinine levels, nor did it induce morphological alterations in the kidneys of the mice [93, 94]. These findings indicate that the use of SpHL-CDDP as a drug delivery system can increase the safety of the drug and improve the therapeutic efficacy of the CDDP-based treatment. Thus, antitumor activity studies were conducted, and the results showed a significant reduction in the tumor volume, a higher tumor growth inhibition ratio, and the complete remission of the tumor in 18.2% of the Ehrlich solid tumor-bearing mice treated with SpHL-CDDP by intravenous route, as compared to the free CDDP treatment [94, 95]. In addition, the survival of animals treated with SpH-CDDP was higher than those treated with free CDDP after i.p. administration in initial or disseminated Ehrlich ascitic tumor-bearing mice [96]. These findings strongly indicate the potential of SpHL-CDDP for future clinical studies.

Oxaliplatin (Figure 8), an analoge of CDDP, has shown a good in vitro and in vivo antitumor effect and a better safety profile than cisplatin. However, the use of oxaliplatin is associated with side-effects which include neurotoxicity, hematologic toxicity and gastrointestinal tract toxicity. In addition, there is a significant risk of grade 3/4 neutropenia to the patients, and the occurrence of nausea and vomiting were generally mild to moderate. Nephrotoxicity is mild, allowing for the administration of oxaliplatin without hydration. Often, severe side effects can be observed, such as tubular necrosis. Furthermore, cellular resistance to free oxaliplatin has been observed, preventing the potential efficacy of free oxaliplatin [97]. Lipoxal® is a liposomal formulation of oxaliplatin made up of hydrogenated soy phosphatidylcholine (HSPC)/DPPC/CHOL/DSPE-PEG. This liposomal formulation containing oxaliplatin has also proven to induce the complete disappearance of human breast cancers in mice after 6 intravenous injections with 4 days intervals at a dose of 16 mg/Kg. On the other hand, the free oxaliplatin at its MTD could only cause shrinkage, not the disappearance of tumors. To estimate the adverse reactions and detect the dose limiting toxicity (DLT), as well as the MTD of Lipoxal®, a Phase I clinical study was conducted. Twenty-seven patients with advanced disease of the gastrointestinal system (stage IV gastrointestinal cancers, including colorectal, gastric, and pancreatic), who had failed previous standard chemotherapy, were treated with escalating doses of Lipoxal® once weekly for 8 weeks. No serious side effects were observed at doses of 100-250 mg/m², whereas at doses of 300 and 350 mg/m² of Lipoxal® monotherapy mild myelotoxicity, nausea and peripheral neuropathy were observed. Gastrointestinal tract toxicity after treatment with Lipoxal® was negligible. Nausea or mild vomiting was observed, but it was eliminated by administering ondansetron. The most common toxicity is peripheral neuropathy at the 300 and 350 mg/m² dose levels. Lipoxal® is well-tolerated and reduces significantly all other side effects of free oxaliplatin, especially myelotoxicity and gastrointestinal-
tinal tract toxicity. These preliminary results showed adequate effectiveness in pretreated patients [98,99].

6.2. Anthracyclines

The anthracyclines, represented by doxorubicin, daunorubicin, and their derivatives (Figure 9), are widely used in the treatment of several hematological and solid tumors and are considered to be a first-line therapy for advanced breast cancer [100]. Although conventional anthracyclines have been extensively used for the treatment of a variety of cancers, they can be associated with the development of substantial cardiotoxicity, which is both cumulative and irreversible. Furthermore, cardiotoxicity can be increased nearly four-fold when these drugs are administered in association with other chemotherapeutic drugs [101]. In this case, the preclinical and clinical studies have focused on the development of liposomal formulations, aimed at decreasing the acute and cumulative cardiotoxicity, in addition to attenuating other drug-related events (e.g. bone marrow depression, alopecia, and nausea) [102].

Forssen and coworkers [103] reported the ability of liposomes containing daunorubicin (DNR), made up of DSPC/CHOL, to accumulate within the P-1798 murine lymphosarcoma and MA16C mammary adenocarcinoma tumor model. The maximum levels of liposome uptake exceeded those achieved by the free drug between 2.5 and 20-fold, which was translated into a 10-fold increase in AUC of tumor exposure to DNR in the P-1798 system. Other investigations also significantly demonstrated increased efficacy and decreased toxicity of liposomes.
containing daunorubicin (DaunoXome®), as compared to free drug in the treatment of acute leukemia and advanced cutaneous T-cell lymphoma [104, 105]. In phase I/II clinical trials, DaunoXome® administration produced a 35-fold increase in the plasma AUC, higher peak plasma concentrations, a smaller distribution volume, and a lower total body clearance, when compared to free DNR [106]. Safety results from the combined phase I and II studies showed DaunoXome® to be especially well-tolerated with minimal myelosuppression, no evidence of cardiac toxicity, and a decrease in the frequency and severity of chemotherapy-related side effects when compared with free DNR. The MTD of liposomal DNR was set at 90 mg/m².

A randomized phase III trial was conducted to compare the safety and efficacy of DaunoXome® with that of a reference regimen of doxorubicin, bleomycin, and vincristine (ABV) as a primary therapy in advanced AIDS-related Kaposi’s sarcoma. DaunoXome® presented an efficacy that was comparable to ABV, presented significantly less alopecia and neuropathy, and showed no evidence of cardiac toxicity [107]. In 1996, DaunoXome® was approved as a first-line therapy for HIV-related Kaposi’s sarcoma by the FDA and the EMA. A European Phase IV study, carried out over a one year period after DaunoXome® had been approved for commercialization, demonstrated the treatment’s good tolerability (absence of cardiotoxicity) and effectiveness. Furthermore, the concomitant administration of highly active antiretroviral treatment (HAART) also proved to be safe [108].

Another commercial product of conventional liposome (Myocet®), in combination with cyclophosphamide, has been approved in Europe as a first-line treatment of breast cancer. This liposome consists of egg phosphatidicholine (EPC)/ CHOL and encapsulated doxorubicin (DXR). Preclinical toxicity studies performed on Beagles demonstrated a better toxicity profile of Myocet®, as compared to free DXR [109]. The ability of Myocet® to locate tumors could be observed in ascitic (L1210 ascitic lymphoma) and solid tumor (murine Lewis lung cancer and B16/BL6 melanoma) models, as reported in findings from Harasym and coworkers [110]. In the case of the solid tumor models, the maximum tumor concentrations were two to three-fold higher for liposomal DXR, as compared to free DXR. For the ascitic model, the maximal level in tumor drug exposure was ten-fold higher for liposomal DXR, as compared to free DXR. These findings supported the choice of Myocet® for clinical studies.

Some studies have shown that the replacement of free DXR by Myocet®, combined with cyclophosphamide, does not result in decreased efficacy parameters, but rather in a significantly reduced risk of cardiotoxicity [56]. A phase III comparison of free DXR with Myocet® in patients with metastatic breast cancer, for instance, demonstrated that, at comparable response rates (RR: 26% for both) and progression-free survival times (PFS: 4 months for both), the incidence of cardiac events (29% vs. 13%) and of congestive heart failure (8% vs. 2%) were significantly lower for Myocet® [102].

Cowens and coworkers [111] carried out a phase I study in 38 patients with refractory solid tumors and demonstrated diminished myelosupression and gastrointestinal toxicity after the intravenous injection of Myocet®, as compared to findings for free DXR at the same dose. The MTD for Myocet® was established as 90 mg/m². A multicentric study including 297 patients with metastatic breast cancer, carried out by Batist and coworkers [112], demonstrated that the combination of Myocet® (60 mg/m²) with cyclophosphamide (600 mg/m²) presents a similar
efficacy and a lower toxicity than does the association of free DXR and cyclophosphamide at the same dose. The cardiotoxicity was dramatically reduced (21% vs. 6%).

The tissue distribution, efficacy, and toxicity of DXR encapsulated in a long-circulating liposomal formulation made up of HSPC/DSPE-PEG<sub>2000</sub>/CHOL (Doxil®/Caelyx®) were also investigated. Therapeutic efficacy studies performed in different animal models demonstrated that Doxil®/Caelyx® was significantly more active than free DXR [113, 114]. A tissue distribution study of this formulation indicated a preferential accumulation within various implanted tumors and human tumor xenografts, with an enhancement of drug concentrations, when compared with free drug, in the tumors. In addition, the cardiac toxicity of Doxil®, as compared to free DXR, was significantly reduced [115].

Doxil®/Caelyx® was the first and is still the only long-circulating liposome formulation to be approved in both the USA and Europe to treat Kaposi’s sarcoma and recurrent ovarian cancer [116, 117]. In association with Velcade® (Bortezomib), this drug is approved by the FDA for the treatment of multiple myeloma. In Europe, this drug is still approved for the treatment of metastatic breast cancer. When compared to free DXR, Doxil® presents a lower plasma clearance (0.1 vs. 25 L/hour for Doxil® and free DXR, respectively) and a small distribution volume (4 vs. 200 L). Doxil® presents two distribution phases: an initial phase with a half-life of 1-3 hours and a second phase with a half-life of 30-90 hours. Its half-life is longer than the free DXR (0.2 hours). Due to this, its cardiotoxicity, myelosuppression, alopecia, and nausea are significantly reduced when compared with an equieffective dose of free DXR. It has also been demonstrated that nearly all circulating drugs (>98%) are used in liposome-encapsulated form, indicating that the pharmacokinetics of liposomal DXR is governed by the liposome carrier and that most of the drug is delivered to the tissues in liposome-associated form [115]. Several studies are currently in progress using Doxil®/Caelyx® to treat other malignancies, such as breast cancer and recurrent high-grade glioma [118-120].

6.3. Other chemotherapeutic agents

Another important drug in cancer therapy is paclitaxel. This is an alkaloid which stabilizes microtubules and inhibits endothelial cell proliferation, motility, and tube formation [121]. Some studies have presented difficulties in the development of liposomes containing paclitaxel due to its hydrophobic nature. Zhang and coworkers [122] developed a liposomal formulation of paclitaxel consisting of 1,2-dioleyl-sn-glycero-3-phosphocholine/CHOL/cardiolipin (LEP-ETU). Therapeutic efficacy studies performed in a mouse xenograft model of human ovarian (OVCAR-3), human lung (A-549), breast (MX-1), and prostate (PC-3) cancer, as compared to the administration of free drugs, demonstrated greater tumor growth inhibition after the administration of liposomal paclitaxel. In addition, toxicology studies have shown that liposomal paclitaxel is less toxic than free paclitaxel. An improved pegylated liposomal formulation of paclitaxel was developed, demonstrating that cytotoxicity in human breast cancer cell lines (MDA-MB-231 and SK-BR-3) of the tested paclitaxel formulation was equipotent after 72 h of incubation, when compared to Taxol®. The pegylated liposomes, as compared to the conventional liposomes, increased the biological half-life of paclitaxel from 5.05 ± 1.52 h to 17.8 ± 2.35 h in rats. Biodistribution studies in a breast cancer xenograft nude
mouse model demonstrated that the uptake of these liposomes significantly increased in tumor tissues after their injection, as compared to Taxol® or the conventional liposomal formulation. Moreover, the pegylated liposome showed a greater tumor growth inhibition effect in in vivo studies [123]. In a study by Strieth et al. (2008) [124], paclitaxel was encapsulated in cationic liposomes composed of dioleyltrimethylammoniumpropane (DOTAP)/DOPC (EndoTAG-1) as a vascular targeting formulation to treat solid tumors and quantified the therapeutic combination with conventional CDDP chemotherapy. This study showed that vascular targeting with EndoTAG-1 increased tumor microvessel leakage, most likely due to vascular damage, and concluded that manipulating the blood-tumor barrier by repeated tumor microvessel targeting using EndoTAG-1 can effectively be combined with tumor cell directed conventional cisplatin chemotherapy.

In a study by Strieth and coworkers, paclitaxel was encapsulated in cationic liposomes composed of dioleyltrimethylammoniumpropane (DOTAP)/dioleoylphosphatidylcholine (DOPC) (EndoTAG-1) as a vascular targeting formulation to treat solid tumors and quantified the therapeutic combination with conventional cisplatin chemotherapy. This study showed that vascular targeting with EndoTAG-1 increased tumor microvessel leakage, most likely due to vascular damage, and concluded that manipulating the blood-tumor barrier by repeated tumor microvessel targeting using EndoTAG-1 can effectively be combined with tumor cell directed conventional cisplatin chemotherapy [124].

Another formulation approved in Europe for lymphomatous meningitis is DepoCyte®, a sustained-release formulation of cytarabine. A randomized study to evaluate the efficacy and safety of this liposomal formulation, in comparison with free drug, was performed in 28 patients with lymphomatous meningitis. While the reference treatment required the administration of free cytarabine biweekly, it could be observed that the administration of DepoCyte® intrathecal maintains cytotoxic concentrations of the drug in the cerebrospinal fluid of most patients for more than 14 days. Response rates (i.e. clearing of cerebrospinal fluid and absence of neurological progression) were significantly higher in DepoCyte®. In addition, the less demanding injection schedule is favorable to the patients’ quality of life. The major adverse events were headache and arachnoiditis, which were often caused by the underlying disease [125]. Another randomized trial compared DepoCyte® with methotrexate in patients with solid tumor neoplastic meningitis. The results showed that median survival was not different, but a greater median time to neurological progression was obtained with DepoCyte®. The frequency and grade of adverse events were comparable between treatments [126]. More recently, a phase II study of intrathecal liposomal cytarabine was performed at the dose of 50 mg in 30 patients with human immunodeficiency virus–non-Hodgkin lymphoma (HIV-NHL) to evaluate the feasibility and activity of prophylaxis. In this study, liposomal cytarabine was well-tolerated, with a headache of grade I to III being the most frequent side effect in 40% of the patients. With a median follow-up of 10.5 months, only 1 (3%) patient developed a combined systemic and meningeal recurrence. The use of liposomal cytarabine allowed for a significant reduction in the number of lumbar injections, as compared to the standard schedules (approximately 50%), improving the patients’ quality of life and reducing their risk of professional exposure [127].
Marqibo®, a DSPC/CHOL encapsulation of vincristine sulfate has targeted, increased, and sustained the delivery of vincristine to tumor tissues. A phase II study evaluated the efficacy and tolerability of Marqibo® as a single agent in patients with multiple relapsed or refractory aggressive non-Hodgkin lymphoma (NHL). In this study, eligible patients again presented relapsed, refractory, or transformed aggressive NHL and prior treatment with at least 2 multiagent chemotherapy regimens. Marqibo® was administered at 2 mg/m², every 2 weeks, for a maximum of 12 cycles or until toxicity or disease progression had been resolved. Marqibo® proved to be an active agent in patients with heavily pretreated aggressive NHL and to be tolerated at approximately twice the dose intensity of standard vincristine [128].

6.4. Recent advances in targeted liposomes

Considering that tumor cells are often characterized by a specific expression pattern of membrane associated proteins, such as receptors, membrane transport systems, or adhesion molecules, cancer therapies that exploit targeting ligands 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. In an attempt to improve the binding and cellular internalization of liposomes in the tumor area, several ligands were attached to the liposome surface, including monoclonal antibodies, folate, transferrin, vasoactive intestinal peptide (VIP), epidermal growth factor (EGF), hyaluronic, galactosides, and condroitin sulphate [129, 130].

The majority of research in this area is related to cancer targeting, which uses a variety of monoclonal antibodies. To target HER2-overexpressing tumors, it was suggested that anti-HER2 long-circulating liposomes be used. Antibody CC52 against rat colon adenocarcinoma CC531 attached to pegylated liposomes provided a specific accumulation of liposomes in rat model of metastatic CC531. A nucleosome-specific monoclonal antibody (mAb 2C5) capable of recognizing various tumor cells through the tumor cell surface-bound nucleosomes significantly improved Doxil®, by targeting to tumor cells, and increased its cytotoxicity both in vitro and in vivo in different testing systems, including the intracranial human brain U-87 tumor xenograft in nude mice. The same antibody was also used to effectively target long-circulating liposomes loaded with an agent for tumor photodynamic therapy (PDT) for both multiple cancer cells in vitro and experimental tumors in vivo, and provided a significantly enhanced elimination of tumor cells under PDT conditions [5].

Previous studies have demonstrated that DXR-loaded long-circulating liposomes prolong circulation in the blood but create a steric barrier that could cause a reduction in the interaction of liposomes with the target cells [131]. In this light, XueMing Li and coworkers [132] prepared DXR-loaded long-circulating liposomes conjugated with transferrin (Tf) and observed that Tf-modified liposomes could be used to enhance the intracellular delivery of anticancer agents, such as cytotoxic drugs, antisense nucleic acids, ribozymes, or imaging agents.

Saccharide molecules represent good models for tumor targeting molecules, as many malignant cells express the lectin, sugar-binding protein. In this context, Song and coworkers [133] investigated the in vitro characteristics of liposomes consisting of HSPC/CHOL/DSPE-PEG₂₀₀₀− disaccharide whose surface had been modified with a disaccharide molecule, sucrose, or
maltose and that were then loaded with DXR. They concluded that disaccharide-modified liposomes may be promising cancer targeting carriers which can enhance intracellular uptake and cytotoxicity of the drug-loaded liposomes by means of lectin-mediated endocytosis.

One approach that has received considerable attention has been the use of folic acid to deliver drugs selectively to folate receptor-expressing cancer cells [130]. Studies of folate-conjugated liposomes containing DNR or DXR showed an increased cytotoxicity of the encapsulated anticancer drugs in various tumor cells [134, 135]. The i.v. administration of anti-tumor-associated glycoprotein (TAG)-72 Polyethylene glycol (PEG)-immunoliposomes showed that they were more effectively located in LS174 T human colon cancer cells than conventional liposomes [136]. It is worth noting that the co-immobilization of PEG and ligands on the same surface liposome can in fact lead to poor target recognition due to a steric hindrance by the hydrophilic corona [137]. Thus, it has been suggested that targeting vectors be attached to the distal end of pegylated phospholipids [138].

Several liposomal formulations of anticancer drugs have also been investigated in preclinical tumor models and many liposomal preparations of anticancer drugs have been approved for cancer chemotherapy or are in advanced stages of clinical development. Some of these products are listed in Table 5.

<table>
<thead>
<tr>
<th>Product</th>
<th>Entrapped Drug</th>
<th>Lipid composition</th>
<th>Company</th>
<th>Therapeutic Indication</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxil®/ Caelyx®</td>
<td>Doxorubicin</td>
<td>HSPC/CHOL/ DSPE-PEG&lt;sub&gt;2000&lt;/sub&gt;</td>
<td>Janssen-Cilag</td>
<td>Kaposi’s sarcoma, recurrent ovarian, multiple myeloma, and metastatic breast cancer</td>
<td>A</td>
</tr>
<tr>
<td>Myocet&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Doxorubicin</td>
<td>EPC/CHOL</td>
<td>Cephalon</td>
<td>Metastatic breast cancer</td>
<td>A&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>DaunoXome&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Daunorubicin</td>
<td>DSPC/CHOL</td>
<td>Galen US</td>
<td>Kaposi’s sarcoma</td>
<td>A</td>
</tr>
<tr>
<td>DepoCyte&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Cytarabine</td>
<td>DOPC/ DPG/CHOL/ TRIOLEIN</td>
<td>Pacira</td>
<td>Lymphomatous meningitis</td>
<td>A</td>
</tr>
<tr>
<td>SPI-077&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Cisplatin</td>
<td>HSPC/CHOL/DSPE-PEG&lt;sub&gt;2000&lt;/sub&gt;</td>
<td>Sequus</td>
<td>Ovarian cancer</td>
<td>P II</td>
</tr>
<tr>
<td>Lipoplatin&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Cisplatin</td>
<td>DPPG/SPC/CHOL/ DSPE-PEG&lt;sub&gt;2000&lt;/sub&gt;</td>
<td>Regulon</td>
<td>Lung cancer</td>
<td>P III</td>
</tr>
<tr>
<td>Aroplatin&lt;sup&gt;®&lt;/sup&gt;</td>
<td>bis-neodecanoate daminocyclohexane platinum</td>
<td>DMPC/DMPG</td>
<td>Anonex</td>
<td>Colorectal, lung, and pancreatic cancer</td>
<td>P II</td>
</tr>
<tr>
<td>LEP-ETU&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Paclitaxel</td>
<td>DOPEC/CHOL/ CARDIOLIPIN</td>
<td>Insys Therapeutics</td>
<td>Breast, lung, ovarian cancer</td>
<td>P II</td>
</tr>
<tr>
<td>EndoTAG-1&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Paclitaxel</td>
<td>DOPEC/DOTAP</td>
<td>MediGene</td>
<td>Breast, pancreatic, and hepatic cancer</td>
<td>P II</td>
</tr>
<tr>
<td>ThermoDox&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Doxorubicin</td>
<td>DPPC/MSPC/DSPE-PEG&lt;sub&gt;2000&lt;/sub&gt;</td>
<td>Celsion</td>
<td>Bone metastasis, breast, and hepatocellular cancer</td>
<td>P II</td>
</tr>
<tr>
<td>Product</td>
<td>Entrapped Drug</td>
<td>Lipid composition</td>
<td>Company</td>
<td>Therapeutic Indication</td>
<td>Status*</td>
</tr>
<tr>
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<td>Marqibo®</td>
<td>Vincristine</td>
<td>DSPC/CHOL</td>
<td>Talloa Therapeutics</td>
<td>Non-Hodgkin’s lymphoma, acute lymphoblastic leukemia, and Hodgkin’s lymphoma</td>
<td>P III</td>
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<tr>
<td>OSI-211® (NX211)</td>
<td>Lurtotecan</td>
<td>HSPC/CHOL</td>
<td>OSI</td>
<td>Ovarian cancer and small cell lung cancer</td>
<td>P III</td>
</tr>
<tr>
<td>LE-SN38®</td>
<td>Irinotecan metabolite SN38</td>
<td>DSPC/CHOL</td>
<td>NeoPharm</td>
<td>Colorectal and lung cancer</td>
<td>P II</td>
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<tr>
<td>INX-0076®</td>
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<td>Sphingomyelin/CHOL</td>
<td>Inex</td>
<td>Ovarian and small lung cancer</td>
<td>P II</td>
</tr>
<tr>
<td>Alocrest®</td>
<td>Vinorelbine</td>
<td>Sphingomyelin/CHOL</td>
<td>Inex</td>
<td>Non-small cell lung cancer and breast cancer</td>
<td>P I</td>
</tr>
<tr>
<td>Oncollin®</td>
<td>Interleukin 2</td>
<td>DMPC</td>
<td>Biomirma USA Inc</td>
<td>Kidney cancer</td>
<td>P II</td>
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<tr>
<td>OSI-7904L®</td>
<td>Thymidylate synthase inhibitor</td>
<td>HSPC/CHOL</td>
<td>OSI</td>
<td>Colorectal cancer</td>
<td>P II</td>
</tr>
<tr>
<td>CPX-351</td>
<td>Cytarabine and Daunorubicin</td>
<td>DSPC/DSPG/CHOL</td>
<td>Celator Pharmaceuticals</td>
<td>Acute myeloid leukemia</td>
<td>P II</td>
</tr>
<tr>
<td>CPX-1</td>
<td>Irinotecan and 5-Fluorouracil</td>
<td>DSPC/DSPG/CHOL</td>
<td>Celator Pharmaceuticals</td>
<td>Advanced colorectal cancer</td>
<td>P II</td>
</tr>
</tbody>
</table>

* A = approved, PI = phase I study, PII = phase II study, PIII = phase III study; a approved by EMA

HSPC, hydrogenated soy phosphatidylcholine; CHOL, cholesterol; DSPE-PEG3000, distearoylphosphatidylethanolamine-polyethylene glycol3000; EPC, egg phosphatidylcholine; DSPC, distearoylphosphatidylcholine; SPC, soy phosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DOPE, dioleoylphosphatidylcholine; DOTAP, dioleoyltrimethylammoniumpropane; DMPC, dimyrystoyl phosphatidylcholine; DSPG, Distearylphosphatidylglycerol; MSPC, Myristoylstearyl phosphatidylcholine

Table 5. Approved and emerging liposome encapsulated anticancer drugs.

7. Future perspectives and challenges

This chapter focused on liposome-based drug delivery systems, which are the most widely used drug nanoparticles in cancer treatments. Basic concepts were presented concerning liposomes and an overview of the clinically used and tested liposomes for the treatment of cancer. It has been demonstrated, based on prior studies, that liposomes offer safety and effectiveness as compared to other conventional treatments.

The greater interest in the development of these sophisticated drug delivery systems is to improve the efficacy and decrease the side effects of new and old anti-cancer drugs. In this context, the optimized pharmacokinetic properties of liposomes, resulting in an improved toxicity profile, is still the main argument for the use of liposomal carriers.

Other new strategies in the biology and pharmacokinetic behavior of liposomes, such as the anti-angiogenic properties of cationic liposomes, as well as the development of immunolipo-
somes or antisense oligonucleotides, also offer a great therapeutic repertoire for these drug delivery systems.

However, despite all progress achieved to date, it is still important to discuss not only the benefits, but also the problems, which remain as a challenge in liposome-based drug delivery systems. As reviewed by Ruenraroengsak and coworkers [139], there are many issues regarding the instability of particles through flocculation and aggregation, their complex flow, and adhesion patterns in the capillary network, the heterogeneity of the access of drugs to specific tumor sites, the diffusion of free drugs, and nanoparticles in tumor tissues as well as in single cells.

The “passive” form of encapsulated drug delivery today is still mostly based on leakage in the tumor microenvironment, followed by the possibility of the cellular uptake of the free drug at the tumor site. As a result, many research groups are working on more “active” therapies that exploit targeting ligands to deliver attached cytotoxic drugs selectively to malignant cells. These ligands specifically recognize and preferentially bind receptors found on the cells of interest, thereby allowing for a more precise delivery method [140].

Although current studies have shown that the use of these targeted nanoparticles as a drug delivery system is a promising strategy to treat human cancers, it is still in its early stage of development. Clinical data using targeted nanoparticles are limited, since most targeted nanoparticles have not yet reached the clinical level. Only a few targeted nanoparticles are currently under clinical investigation. In addition, advanced imaging techniques are essential, especially in small animals, to verify the true extent of tumor and target localization [139].

In sum, liposomes provide many targeting strategies and have shown a promising future as a new generation of cancer therapeutics. Certain critical questions and many obstacles still remain, which present specific limitations to their overall efficacy. However, as soon as more clinical data becomes available, further understanding will certainly lead to a more rational design of optimized liposomes with improved selectivity, efficacy, and safety in cancer treatment [140].

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References


[28] Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, Marks JD, Benz CC, Park JW. Antibody targeting of long-circulating lipidic nanoparticles does
not increase tumor localization but does increase internalization in animal models. Cancer Research 2006;66(13) 6732-6740.


Fedier A, Poyet C, Perucchini D, Boulikas T, Fink D. MLH1-deficient tumor cells are resistant to lipoplatin, but retain sensitivity to lipoxal. Anticancer Drugs. 2006;17(3) 315-323.


Jehn CF, Boulikas T, Kourvetaris A, Kofla G, Possinger K, Lüftner D. First safety and response results of a randomized phase III study with liposomal platin in the treat-
ment of advanced squamous cell carcinoma of the head and neck (SCCHN). Anti-
cancer Research 2008;28(6B) 3961-3964.

[88] Stathopoulos GP, Antoniou D, Dimitroulis J, Michalopoulou P, Bastas A, Marosis K,
Stathopoulos J, Provata A, Yiamboudakis P, Veldakis D, Lolis N, Georgatou N, Tou-
bis M, Pappas Ch, Tsoukalas G. Liposomal cisplatin combined with paclitaxel versus
cisplatin and paclitaxel in non-small-cell lung cancer: a randomized phase III multi-

[89] Stathopoulos GP, Boulikas T. Lipoplatin formulation review article. Journal of Drug
Delivery 2012;2012 581363.

[90] Júnior ADC, Mota LG, Nunan EA, Wainstein AJA, Wainstein APDL, Leal AL, Cardo-
so VN, Oliveira MC. Tissue distribution evaluation of stealth pH-sensitive liposomal
cisplatin versus free cisplatin in Erlich tumor-bearing mice. Life Sciences 2007;80(7)
659-664.

[91] Chauffert B, Favoulet P, Polycarpe E, Duvillard C, Beltram JL, Bichat F, Rat P,
Genne P, Benoit L. Rationale supporting the use of vasoconstrictors for intraperito-
neal chemotherapy with platinum derivatives. Surgeon Oncology Clinics of North

[92] Araújo JG, Mota LG, Leite EA, Maroni Lde C, Wainstein AJ, Coelho LG, Savassi-Ro-
cha PR, Pereira MT, de Carvalho AT, Cardoso VN, De Oliveira MC. Biodistribution
and antitumoral effect of long-circulating and pH-sensitive liposomal cisplatin ad-
ministered in Ehrlich tumor-bearing mice. Experimental Biology and Medicine (May-
wood) 2011;236(7) 808-815.

[93] Leite EA, Giuberti CS, Wainstein AJ, Wainstein AP, Coelho LG, Lana AM, Savassi-
Rocha PR, De Oliveira MC. Acute toxicity of long-circulating and pH-sensitive lipo-
somes containing cisplatin in mice after intraperitoneal administration. Life Sciences
2009;84(19-20) 641-649.

[94] Leite EA, Lana AM, Junior AD, Coelho LG, De Oliveira MC. Acute toxicity study of
cisplatin loaded long-circulating and pH-sensitive liposomes administered in mice.
Journal of Biomedical Nanotechnology 2012;8(2) 229-239.

[95] Leite EA. Avaliação da Toxicidade Aguda e Atividade Antitumoral de Lipossomas
pH-sensíveis de circulação prolongada contendo cisplatina. PhD Thesis. Universi-
dade Federal de Minas Gerais; 2010.

[96] Maroni, LC, Silveira ACO, Leite EA, Melo MM, Ribeiro AFC, Cassali GD, Souza CM,
Fagundes EMS, Caldas IR, Araújo MSS, Filho OAM, Oliveira MC, Carvalho AT. An-
titumor effectiveness and toxicity of cisplatin loaded long-circulating and pH-sensi-
tive liposomes against Ehrlich ascitic tumor. Experimental Biology and Medicine


