Liposomes are phospholipid bilayered spheres that have become increasingly interesting as chemotherapeutic agents nanocarriers. Mainly, antitumor drugs encapsulation minimizes their toxicity and increases accumulation at the target site(s), thus leading to a higher therapeutic index.

The aims of the present review are:
- To introduce the liposomes formation and pharmacokinetics.
- To enumerate some antibody derivatization and conjugation strategies.
- To analyze advances in chemotherapeutic immunoliposomes under in clinical level.
- To mention some immunoliposome applications.

Liposomal nanoparticles can passively target tumors owing to the enhanced permeability and retention (EPR) effect. However, nowadays liposomes can be optimized enhancing the ability to specifically recognize and bind target tissue by means of the high affinity interaction of some molecules.

Antibodies coupling to the liposomal surface for active targeting is an important strategy as known as immunoliposomes.

Several types of liposomes are currently approved by the US Food and Drug Administration (FDA) although immunoliposomes still are under clinical trial phases. Here there are some of the advantages and disadvantages of immunoliposomes as nanocarriers:

Advantages
- Enhanced therapeutic index
- Drug loading versatility
- Specific targeting
- Increased half-life circulation
- Intracellular drug delivery

Disadvantages
- Sometimes immunogenic
- High cost
- Difficult barrier penetration

Liposome formation and pharmacokinetics

Liposomes are the result of the hydration of dry lipid films. These enclosed spherical vesicles are flexible, biocompatible, and biodegradable, easy to prepare and have small particle size yet within high loading capacity. In general, liposomal production includes these 5-stages:

1. Drying down lipids from organic solvent:
   - Phosphatidylcholine
   - Cholesterol
   - Others

2. Liquid dispersion in aqueous media

3. Drug loading:
   - During formation
   - After formation
   - Passively
   - Actively

4. Liposome purification

5. Product stability analysis

The main advantage of liposomes is their versatility in drug delivery. They can carry a variety of drugs, including small molecules, macromolecules such as proteins, and nucleic acids. They can be formulated to target specific tissues and organs, providing a controlled release of the therapeutic agent.

Table 1. Liposome characteristics

<table>
<thead>
<tr>
<th>Liposome type</th>
<th>Half-life circulation</th>
<th>Solubility</th>
<th>Pharmacokinetics</th>
<th>Targeting</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>First generation liposomes</td>
<td>Short (RES)</td>
<td>Low</td>
<td>Low</td>
<td>Passive</td>
<td>Uncontrolled</td>
</tr>
<tr>
<td>Second generation PEGylated liposomes</td>
<td>Long</td>
<td>High</td>
<td>Dose-dependent</td>
<td>Passive</td>
<td>Uncontrolled</td>
</tr>
<tr>
<td>Third generation PEGylated liposomes</td>
<td>Medium</td>
<td>High</td>
<td>Dose-independent</td>
<td>Active</td>
<td>Intraocular</td>
</tr>
</tbody>
</table>

Derivatization and conjugation strategies

Antibody molecules are composed by the fragment crystallizable (Fc) and antigen-binding fragment (Fab) that contains the light chain and a portion of the heavy chain. The Fab fragment is further divided into the variable fragment (Fv), the smallest fragment that retains antigen binding as help via linker with both the heavy and light chains. The two chains of the Fab fragment are held together by a disulfide bond whereas the two chains of the Fv are coupled either by a flexible polypeptide linker or by a disulfide bond (suFc).

Antibody molecules can be generated through:
- Chemical processing
- Reducing agents or enzymatic degradation (pepsin and papain).
- Genetic fusion
- Phage display.

Requirements of the antigen component to take into consideration:
- Specificity and binding affinity.
- Lack of immunogenicity.
- Conservation in systemic circulation.
- Internalization ability.
- Production cost.

For this requirements, and specially for the RES rapid clearance due to the immunogenicity of the fragment crystallizable (Fc) chain, nowadays Fab, F(ab)2, Fv and suFv antibody fragments are becoming more popular rather than whole antibodies.

For the antibody attachment to the liposomes, the method most extensively used is the maleimide-PEG. This is based on the conjugation by means of two-thiol bonds between thiolated antibodies and liposomes containing Maleimide(polyethylene-glycol)distearoylphosphatidylethanolamine (Mal-PEG-DISTE).

Figure 1. Schematic representation of different antibody fragments.

Table 2. Antibody targeted liposomes for cancer treatment in clinical trials phase.

<table>
<thead>
<tr>
<th>Product</th>
<th>Drug</th>
<th>Drug target</th>
<th>Antibody</th>
<th>Formulation</th>
<th>Condition</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxil</td>
<td>Doxil</td>
<td>Doxil</td>
<td>Doxil</td>
<td>Doxil</td>
<td>Doxil</td>
<td>Doxil</td>
</tr>
<tr>
<td>Abraxane</td>
<td>Abraxane</td>
<td>Abraxane</td>
<td>Abraxane</td>
<td>Abraxane</td>
<td>Abraxane</td>
<td>Abraxane</td>
</tr>
<tr>
<td>SGT-01</td>
<td>SGT-01</td>
<td>SGT-01</td>
<td>SGT-01</td>
<td>SGT-01</td>
<td>SGT-01</td>
<td>SGT-01</td>
</tr>
</tbody>
</table>

Conclusion and future directions

Despite the little clinical development to date, results suggest that antibody-coupled liposomes appear to be a promising strategy for targeted chemotherapy:
- Immunoliposomal formulations have shown to reduce side effects.
- Targeted liposomes enhance local tumor cytotoxicity via cell-specific dependent endocytosis and the bystander effect and overcome multiresistance.
- suFv coupling is the preferred conjugation strategy since it offers slower clearance, lower production cost and the ability to engineer suFv with desired affinity and specificity using phage display.

Future directions to take into consideration:
- Pharmacokinetics of drug unloading should be studied thoroughly for a better understanding of immunoliposomes preparation methods to achieve long circulation time, efficient tumor targeting and optimal release profiles of chemotherapeutic agents.
- Standards for parameters analysis in vitro and in vivo models are required as well-defined criteria and comprehensive guidelines for both regulatory approval and large-scale industrial production.
- Major research effort should be undertaken to improve immunoliposomal properties and to find the most relevant targets on tumor cells, thus creating a potential new avenue for safe and effective targeted delivery of anticancer drugs in patients.

References

Only recent references are cited. A detailed bibliography is available upon request for the committee.