Immunoliposomes State Of The Art: Pharmacokinetics Of A Novel Drug Delivery System For Cancer Treatment

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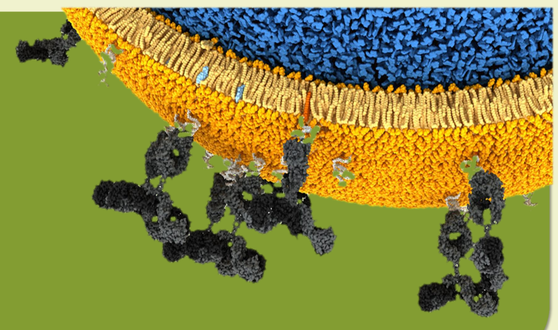

Introduction

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 immunoliposomes 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 tissues by means of the high affinity interaction of some molecules.

Passive targeting → EPR effect

- Extensive angiogenesis
- Defective vascular architecture
- Impaired lymphatic drainage
- Increased production of permeability mediators

Active targeting → Specific binding

- Whole antibodies or some fragments
- High affinity molecules (i.e. folate or transferrin)

Antibodies coupling to the liposomal surface for active targeting generates what is known as immunoliposome.

Several types of liposomes are currently approved by the US Food and Drug Administration (FDA) although immnoliposomes still are under clinical trial phases.

Here there are some of the advantages and disadvantages of immunoliposomes as nanocarriers:

Advantages

- Enhanced therapeutic index
- Site-avoidance mechanism
- 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 lipids 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:

Drying down lipids from organic solvent:
- Phosphatidylethanolamine
- Cholesterol
- Others

→ **Lipid dispersion in aqueous media** → **Drug loading**
- During formation
- After formation
  - Passively
  - Actively

→ **Liposome purification** → **Product viability analysis**

First generation liposomes suffered of a short half-life due to rapid elimination from the circulation by the cells of the reticuloendothelial system (RES) as they are recognized as foreign particles. For this reason, the surface of the liposome membrane was remodelled using hydrophilic polymers, usually polyethylene glycol (PEG), providing an additional surface hydration layer. The surface hydration, also known as PEGylation, enlarges liposomal circulation half-life and confers dose-independent pharmacokinetics. Finally PEGylated liposomes were attached to antibodies to gain specific targeting and clathrin-dependent endocytosis .

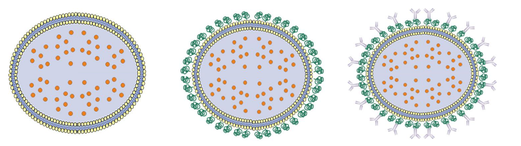

Table 1. Liposome generations characteristics

	First generation Liposomes	Second generation PEGylated liposomes	Third generation PEGylated immunoliposomes
Half-life circulation	Short (RES)	Long	Medium
Solubility	Low	High	High
Pharmacokinetics	Dose-dependent	Dose-independent	Dose-independent
Leakage	High	Low	Low
Targeting	Passive	Passive	Active
Delivery	Uncontrolled	Uncontrolled	Intracellular

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 activity via contacts 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 (scFv).

Antibody moieties can be generated through:

- Chemical processing → reducing agents or enzymatic digestion (pepsin and papain).
- Genetic formation → phage display.

Requirements of the antibody component to take into consideration:

- Specificity and binding affinity.
- Lack of immunogenicity.
- Conservation in systemic circulation.
- Internalization ability.
- Production cost.

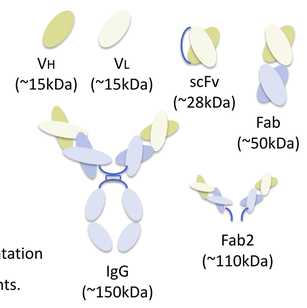

Figure 1. Schematic representation of different antibody fragments.

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 scFv 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. It is based on the conjugation by means of thio-ether bonds between thiolated antibodies and liposomes containing Mal-PEG-polyethylene-glycol-distearoylphosphatidylethanolamine (Mal-PEG-DSPE). This method results in stable covalent bonds and has high coupling efficiency, especially if applied to Fab' or scFv fragments containing reduced sulfhydryl groups.

Materials and Methods

- **Scientific literature search on PubMed, Web of Science database and Wiley Online Library:** recent general reviews and papers of immunoliposomes for cancer treatment were selected according to their quality and data of publication.
- **Pharmaceutical literature research on Clinical trials database, Food and Drug Administration and European medicines agency:** only marketed or under clinical phases pharmaceutical formulations were analyzed.

Immunoliposomes under clinical trials

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

Product	Drug	Target	Ab fragment	Formulation	Condition	Status
MCC-465	DXR	GAH	F(ab')2	GAH F(ab')2-Mal-DPPC	Gastric and colorectal cancer	Phase I
anti-EGFR-ILs-dox	DXR	EGFR	Fab'	C225 Fab'- Mal-PEG-DSPE	Solid tumor	Phase I
MM-302	DXR	HER2	scFv	F5cys-MP-PEG-DSPE	Breast cancer	Phase II/III
SGT-53	P53 DNAp	TfR	scFv	TfRscFv- DOTAP:MPB-DOPE-HoKC	Solid tumors, pancreatic cancer and glioblastoma	Phase I/II
SGT-94	RB94 DNAp	TfR	scFV	TfRscFv-DOTAP:MPB-DOPE-HoKC	Solid tumors	Phase I

Abbreviations: DXR (doxorubicin), DNAp (DNA plasmid), GAH (goat anti-human), EGFR (epidermal growth factor receptor) , HER2 (human EGFR 2), TfR (transferrin receptor), C225 (anti-EGFR antibody), F5cys (anti-HER2 scFv), maleimidated dipalmitoyl-phosphatidylethanolamine (Mal-DPPC), Mal-PEG-polyethylene-glycol-distearoylphosphatidylethanolamine (Mal-PEG-DSPE), MP-PEG-DSPE (maleimidopropylamide-PEG-DSPE), DOTAP (1,2-Dioleoyl-3-trimethylammonium propane), MPB-DOPE (N-maleimido-phenylbutyrate-dioleoylphosphatidylethanolamine), HoKC (histidylated oligolysine peptide).

MCC-465

- Dose limiting toxicity is myelosuppression and appetite loss whereas neither palmar–plantar erythrodysesthesia nor cardiotoxicity are observed.
- Most patients treated stabilize the disease, although no objective antitumor response is seen.

C225-ILS-DOX

- EGFR expression in tumors is up to three orders of magnitude higher than EGFR expression in normal tissue.
- Tumor citotoxicity is higher as a result of the intracellular unloading and the bystander effect.

MM-302

- Advanced/metastatic breast cancer patients show enhanced treatment efficacy.
- 64Cu marked MM-302 was tested for PET/CT imaging.

SGT-53 and SGT-94

- MPB-DOPE and HoKC facilitate DNA unloading in endosome acidic conditions.
- SGT-53 and SGT-94 are loaded with p53 and Rb94 (retinoblastoma) DNA plasmids respectively that aim to restore the loss of these tumor suppressor functions present in most human cancers.
- Both formulations are well tolerated, exhibit anticancer activity, and also supply evidence of targeted transgene expression in metastatic tumors.

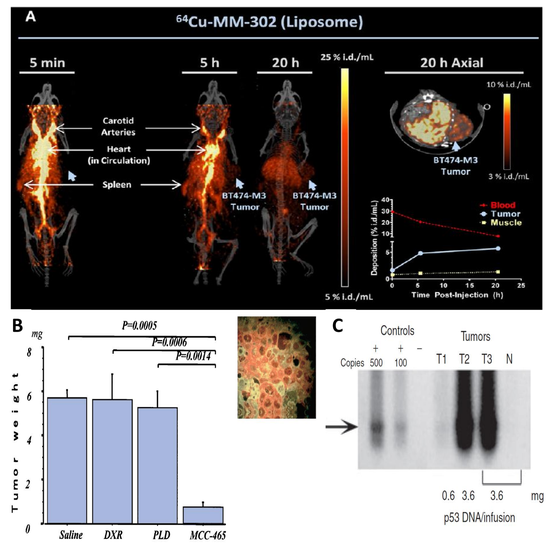

Figure 2. A) 64Cu-MM-302 enhanced targeting to HER2-overexpressing breast carcinoma (BT474-M3)**.** Adapted from Lee H et al., 2015. **B)** *In vivo* antitumor activities of DXR (doxorubicin), PLD (PEGylated liposome DRX), and MCC-465 in mice bearing a colorectal adenocarcinoma xerograph. Adapted from Hamaguchi T et al., 2004. **C)** p53 transgene tumor expression. Adapted from Senzer N et al., 2013.

Other applications

Immunoliposomes are currently being used for drug delivery, diagnosis, imaging, cosmetics, gene therapy and immunotherapy.

Conclusions 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 clathrin-dependent endocytosis and the bystander effect and overcome multidrug resistance.
- scFv coupling is the preferred conjugation strategy since it offers slower clearance, lower production cost and the ability to engineer scFv with the 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 of *in vitro* and *in vivo* models are required as well 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 relevant references are cited below. A detailed references list is available upon request for the committee:

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