International Journal of Drug Development & Research
| Oct-Dec 2010 | Vol. 2 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
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CLINICAL PHARMACOKINETIC ASPECTS OF STEALTH LIPOSOMES: A REVIEW

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ABSTRACT

Stealth liposomes are long-circulating liposomes with inclusion of the synthetic polymer poly-(ethylene glycol) (PEG) in liposome composition. The presence of PEG on the surface of the liposomal carrier has been shown to extend blood-circulation time while reducing mononuclear phagocyte system uptake. Further these liposomes exhibit increasing drug stability and solubility, lowering toxicity, increasing half-life, decreasing clearance and immunogenicity. Sterically stabilized vesicles can act either as long circulating micro reservoirs or tumour (or site of inflammation and infection) targeting vehicles. The former applications require larger liposomes (0.2µm) while the latter one is due to the ability of small vesicles to leave the blood circulation. The altered biodistribution of stealth liposomes, in addition to the accumulation at the sites characterised with porous blood capillaries, such as in tumors, inflammations, and infections. A pharmacogenomic approach for delivery of siRNA to cells is the use of liposomes as targeted delivery vehicles. Stealth technology summarizes pre-clinical and clinical data relating to the principal liposome formulations, encapsulating active molecules, with high target efficiency and activity. Further these liposomes offer improvements in bioreclamation and various monitoring and analytical-diagnostic applications. The paper reviews the clinical aspects of these liposomes with longer therapeutic half lives in diseases like Reconstitution of membrane proteins into artificial membranes, model biological membranes, cell function, fusion, recognition , pharmaceutics studies of drug action , medicine drug-delivery and medical diagnostics, gene therapy and there extensive use in the pharmaceutical industry.

Key Words: *Stealth liposomes, long-circulating liposomes, poly-(ethylene glycol) (PEG), mononuclear phagocyte system, siRNA, drug-delivery.*

INTRODUCTION

The present era of clinical research and modern drug delivery focuses primarily on reducing the toxicological behavior and increasing the therapeutic value of a drug. For this very purpose site specific delivery of drugs in the form of sterically protected nanoparticles and liposomes came into existence. The general concepts of liposomes elucidate a tiny bubble (vesicle), made out of the same material as a cell membrane (lipid bilayer).Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. The lipids in the plasma membrane are chiefly phospholipids like phosphatidylethanolamine and phosphatidylcholine. [1] Phospholipids are amphiphilic with the hydrocarbon tail of the molecule being hydrophobic; its polar head hydrophilic. As the plasma membrane faces watery solutions on both sides, its phospholipids accommodate this by forming a phospholipid bilayer with the hydrophobic tails facing each other. Liposomes can be composed of naturally-

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derived phospholipids with mixed lipid chains (like phosphatidylethanolamine), or of pure surfactant components like DOPE (dioleoylphosphatidylethanolamine).[2] Liposomes, usually but not by definition, contain a core of aqueous solution; lipid spheres that contain no aqueous material are called micelles, however, reverse micelles can be made to encompass an aqueous environment. [3]

LIPOSOMAL CLINICAL APPLICATIONS IN PHARMACEUTICAL INDUSTRY

The basic use of stealth liposomes is to increase the therapeutic half life of the drugs targeted to various organs, but before that it is essential to understand the general concept of drug targeting in liposomal context. Applications of liposomes in pharmacology and medicine can be divided into therapeutic and diagnostic applications of liposomes containing drugs or various markers, and their use as a model, tool, or reagent in the basic studies of cell interactions, recognition processes, and of the mode of action of certain substances. [4] The basic applications include:

1. An eminent property of liposomes is to solubilize drugs like Amphotericin B. These drugs are used in the treatment of fungal infections orally or topically for which they must be soluble in lipid layer, hence liposomes are contemplated as effective drug delivery systems.[5]
2. Certain drugs like doxorubicin have an adverse effect of causing cardiotoxicity, whereas Amphotericin causes nephrotoxicity. The generic liposomal coating decreases there toxic action. [6]
3. The use of sustained release dosage form has being of novel importance for Systemic antineoplastic drugs, hormones, corticosteroids, drug depot in the lungs for there extensive use in Cancer biotherapeutics.[7]
4. Drug degradation of nucleic acid and tissue mediators (like Cytosine arabinoside, interleukins used in cancer treatment) by phagocytes is unwanted. The use of liposomal coatings in drug targeting helps in Drug protection. [8]
5. Reticular endothelial system targeting is achieved for immunomodulators, vaccines, antimalarials for macophage-located diseases and cancer. Further extravasation by liposomes in leaky vasculature of tumors, inflammations is useful for cancer and bacterial infections. [9]

COATINGS IN STEALTH LIPOSOMES

Further advances in liposome research have been able to allow liposomes to avoid detection by the body's immune system, specifically, the cells of reticuloendothelial system (RES). These liposomes are "stealth liposomes", and are constructed with PEG (Polyethylene Glycol) studding the outside of the membrane. The PEG coating, which is inert in the body, allows for longer circulatory life for the drug delivery mechanism. [10] A significant step in the development of long-circulating liposomes came with inclusion of the synthetic polymer poly-(ethylene glycol) (PEG) in liposome composition. The presence of PEG on the surface of the liposomal carrier has been shown to extend blood-circulation time while reducing mononuclear phagocyte system uptake (stealth liposomes). Poly-ethylene glycols have been used to derivatize therapeutic proteins and peptides, increasing drug stability and solubility, lowering toxicity, increasing half-life, decreasing clearance and immunogenicity.

Surface modification of liposomes with PEG can be achieved in several ways:

1. Physically adsorbing the polymer onto the surface of the vesicles.
2. Incorporating the PEG-lipid conjugate during liposome preparation.

3. Covalently attaching reactive groups onto the surface of preformed liposomes.[11]

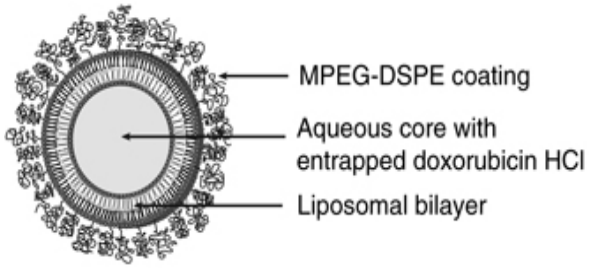
The presence of PEG on the liposome surface provides a strong inter bilayer repulsion that can overcome the attractive Van der Waals forces, thus stabilizing liposome preparations by avoiding aggregation. In particular, from X-ray analysis of bilayers incorporating PEG1900-lipid, their research showed that the grafted polymer moiety extends about 50Å from the lipid surface and gives rise to strong inter-membrane repulsive forces. [12]

Poly amino acids (PAAs) have been evaluated as coating polymers for long-circulating liposomes. The pharmacokinetics of PAA-coated liposomes assessed in rats prolongs the circulation times, comparable to those reported for poly (ethylene glycol) (PEG) - liposomes. [13]

Thermo sensitive liposomes (TSL) can release drugs upon heat. The optimum 1, 2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-PEG_{2000} (DSPE-PEG_{2000}) concentration in stealth TSL to improve content release efficiency under mild hyperthermia (HT) is also an important application. TSL are used with DSPE-PEG_{2000} from 1 to 10 mol%, around 80 nm in size. [14]

Targeting liposomes to the lymph nodes for administration of antitumor, antibacterial, and antiviral drugs is of particular interest, and the pharmacokinetics and biodistribution of PEG-DSPE liposomes have been examined after subcutaneous administration. Subcutaneous administration of PEGylated liposomes also appears interesting; this administration route could become very important, especially for targeting to the lymph nodes and to achieve sustained drug release in vivo. [15]

Furthermore, the use of subcutaneously administered liposomes in the field of vaccination and rheumatism, with the aim of prolonging release of antigens or forming a local drug depot, is also in the focus of interest. PEG-coating on liposomal surface may have a negative effect on lymph node uptake (reduced adsorption by phagocytosis). [16]



A basic sketch of a stealth liposome

STEALTH LIPOSOMES IN CANCER

Stealth liposomes are important in cancer treatment for their passive targeting effect, which may lead to preferential accumulation in tumor tissue, but this phenomenon is not fully understood: Stealth liposomes are able to lodge in the interstitial spaces among tumor cells but, once in the tumor area, they locate in the extracellular fluid surrounding the tumor cell without entering it. Thus, to deliver the active form of an anticancer agent, such as doxorubicin or cisplatin, the drug must be released from the liposomes into the tumor extracellular fluid and then diffuse into the cell. [18-19]

1. PEGylated liposomal doxorubicin (PLD) (DOXIL/ Caelyx) was the first and is still the only stealth liposome formulation to be approved in both the USA and Europe for treatment of Kaposi's sarcoma and recurrent ovarian cancer. Due to its pharmacokinetic behavior, cardiotoxicity, myelosuppression, alopecia and nausea are significantly decreased with PLD compared with an equi-effective dose of conventional doxorubicin. These bio-distribution characteristics also make skin treatment of localized cancers such as Kaposi's sarcoma possible; on the other hand, due to its reduced clearance, the palmar-plantar skin reaction and stomatitis/mucositis are the chief dose-related toxicities of PLD.[20]

2. Another stealth liposome formulation is SPI-077™ (Alza Corporation, Mountain View, CA, USA), in which cisplatin is encapsulated in the aqueous core of sterically stabilized liposomes (fully hydrogenated soy HSPC, CHOL, and DSPE-PEG). The stealth behavior of these compounds is evident from their apparent half-life of approximately 60–100 hours.[21]
3. S-CKD602 (Alza Corporation), a PEGylated stealth liposomal formulation of CKD-602, which is a semi-synthetic analog of camptothecin, is submitted for a Phase I trial.[22]
4. Lipoplatin™ (Regulon Inc. Mountain View, CA, USA) is another liposomal cisplatin formulation composed of dipalmitoyl phosphatidyl glycerol (DPPG), soy PC, CHOL, and mPEG2000-DSPE. Its reported half-life is 60–117 hours, depending on the dose.[23]
5. Mitoxantrone (Novantrone®, Wyeth Lederle, and Madison, NJ, USA) is a drug used for the treatment of acute myeloid leukemia, multiple sclerosis, and prostate cancer. Despite the promising early results of a PEGylated mitoxantrone formulation the only currently existing formulations with lipids and cardiolipine are in clinical trials (as described above).[24]
6. Small unilamellar stealth monensin liposomes (SMLs) were prepared from multi lamellar liposomes (MLVs). The MLVs were prepared by using dipalmitoyl phosphatidylcholine (DPPC), cholesterol,distearoyl-glycerophospho- ethanolamine coupled to poly ethylene glycol (DSPE–PEG) and stearylamine in the molar ratio of 10:5:1.4:1.4 (32.8 mM total lipid). These stealth liposomes acted as a potentiator of Adriamycin in cancer treatment.[25]
7. Stealth liposomes are effective vehicles for drugs, genes and vaccines and can be easily modified with proteins, antibodies, and other appropriate ligands, resulting in attractive formulations for targeted drug delivery. Doxorubicin-loaded stealth liposomes (Tf-SL-DOX) by film dispersion followed by ammonium sulphate gradient method are conjugated Tf to the liposome surface by an amide bound between DSPE–PEG_{2000}–COOH and Tf. The results of the intracellular uptake indicate that Tf-modified SL was able to enhance the intracellular uptake of the entrapped DOX by HepG2 cells compared to SL-DOX. [26] Further Tumor accumulation and therapeutic activity of Stealth liposomes loaded with doxorubicin (DXR) were examined in Balb/c nude mice xenografts inoculated subcutaneously with the human small cell lung cancer (SCLC) cell line, H69. Mice were treated with non-targeted liposomes (SL) or liposomes targeted with antagonist G coupled to the liposome surface (SLG). The therapeutic efficacy of DXR-containing SL or SLG was significantly improved over free DXR, but SLG did not improve anti-tumor efficacy relative to SL. Stealth liposomes containing DXR have potential as a therapy against human SCLC tumors. [27]

STEALTH LIPOSOMES MEDIATORS IN OTHER DISEASES

These long circulating liposomes are also useful at preliminary stages in drug targeting for diseases like immunotoxicity, tuberculosis, and even in studying the epitope of HIV virus. These are further exclaimed as:

1. The stealth liposomes of the carboxylic ionophore, monensin was conjugated to anti-My9 monoclonal antibody (targeted against CD 33 antigen) by a disulfide linkage with almost full retention of immunoreactivity. Hence it further contemplated to enhance the in-vitro cytotoxicity of immunotoxin by several folds using antibody-conjugated monensin liposomes.[28]
2. Liposomes with enhanced affinity towards lung tissue were prepared for the development of more effective chemotherapy

against tuberculosis. Modification of surface of stealth liposomes by tagging *O*-stearyl amylopectin (O-SAP) resulted in the increased affinity of these liposomes towards lung tissue of mice. Liposomes containing egg phosphatidylcholine (ePC), cholesterol (CH), dic etylphosphate (DCP), O-SAP and mono sialogangliosides (GM_1)/distearylphosphatidyl-ethanolamine-poly(ethylene glycol) 2000 (DSPE-PEG 2000) were found to be most stable in serum.[29]

3. PEG grafted liposomes carrying epitopes on their surface showed enhanced adjuvanticity than liposomes carrying epitopes for elicitation and prolongation of immune response to an antigenic epitope of gp41, a transmembrane protein of HIV-1. The multiples of epitope were incorporated onto the surface of liposomes by conjugating them with phosphatidylethanolamine that was used in the formulation of liposomes at an optimized ratio. PEG grafted epitopes carrying liposomes showed about two times higher immune response and prolonged persistence of antibodies than that of liposomes carrying epitopes without PEG moieties. [30]

PHARMACOGENOMIC ASPECTS OF STEALTH LIPOSOMES

RNA interference (RNAi) has been one of the most important pharmacogenomic concepts in functional genomics of the past decade, having enormous potential as a tool for analysing gene function, and for developing novel therapeutics based on gene silencing. The finding that the introduction of small interfering RNA (siRNA) duplexes (19–23 nucleotides long) into cells can induce a sequence-specific degradation and inhibition in the expression of the targeted mRNA, seems set to revolutionize the potential for effective use of RNAi for both research and therapeutic applications. SiRNAs act catalytically to mediate cleavage of the targeted mRNA, and are very potent. Many human diseases including cancer as well as metabolic and neurodegenerative diseases have an underlying genetic basis or can be remedied by silencing specific genes. The systemic administration of siRNA is costly and may result in unwanted off-target effects, highlighting the importance of being able to target siRNAs to specific cells. [31]

An attractive approach for delivery of siRNA to cells is the use of liposomes as targeted delivery vehicles [25]. Cationic lipids can form complexes with nucleic acids, and are widely used as components of liposomal reagents used for the transfection of cells with DNA and siRNAs in vitro [32]. Such liposomes often contain a large proportion of one or more cationic lipids, e.g. 1, 2-dioleoyl-3-trimethylammonium-propane (DOTAP), and the zwitterionic helper lipid 1, 2-dioleoyl-phosphatidylethanolamine (DOPE). Cationic lipids interact efficiently with nucleic acids, forming lipid/nucleic acid complexes (lipoplexes). Unfortunately, whilst cationic liposomes can facilitate transfection of cells *in vitro*, their propensity to aggregate and to interact non-specifically with negative charges on the surface of cells makes their targeted delivery to specific cells *in vivo* difficult [33] and [34]. As an alternative to using cationic liposomes, the encapsulation of siRNA into neutral stealth liposomes seems an excellent strategy for delivering siRNAs to cells *in vivo*. Such liposomes are generally non-toxic, avoid non-specific interactions with blood components and the lipid barrier protects the encapsulated siRNA cargo from rapid degradation by serum nucleases. Despite progress towards the efficient encapsulation of drugs and nucleic acids into neutral stealth liposomes a convenient method of targeting has been lacking. [35]

Potential applications of this technology, therefore, include the development of gene therapy to correct or modify cell function in genetic and other disorders such as cancer. Also, an ability to target siRNAs to immune cells such as dendritic cells in vivo could have enormous potential for manipulating immune function and the development of more effective vaccines and cancer immunotherapies. For example, the targeting of siRNAs to Dendritic cells could be useful in strategies to overcome tumour-induced immunosuppression [36], or for altering immunity as a treatment for autoimmune diseases[37] and [38]. The approach for targeting siRNA delivery could thus potentially be developed for use in many therapeutic applications.

CONCLUSION AND DISCUSSIONS

The paper hence reviews the use of stealth liposomes as a much more dynamic and therapeutically useful endeavor. The basic industrial applications of liposomes include applications with liposomes as the solubilizers for difficult-to-dissolve substances, dispersants, and sustained release systems, delivery systems for the encapsulated substances, stabilizers, protective agents, microencapsulation systems and microreactors being the most obvious ones. But the stealth liposomes have inevitable importance in increasing the half lives of drugs in case of drug delivery in basic sciences such as Reconstitution of membrane proteins into artificial membranes, Model biological membranes, cell function, fusion, recognition , studies of drug action , Medicine Drug-delivery and medical diagnostics, gene therapy makes these carriers suitable for extensive use in the pharmaceutical industry. These long circulating liposomes are also useful at preliminary stages in drug targeting for diseases like immunotoxicity, tuberculosis, and even in studying the epitope of HIV virus. The development of gene therapy to correct or modify cell function in genetic and other disorders such as cancer is an ability to target siRNAs. Further a comparison can be drawn from the following table showing a comparative evaluation of traditional liposomes and stealth liposomes.

S. No.	PROPERTIES	CONVENTIONAL LIPOSOMES	STEALTH LIPOSOMES
1.	Mononuclear phagocyte system (MPS)	These are generally reacted and degraded by the MPS and hence are having short duration of action.	These liposomes substantially avoid the uptake by the MPS.
2.	Coatings (generally used; modifications are there.)	Naturally-derived phospholipids with mixed lipid chains (like phosphatidylethanolamine), or of pure surfactant components like DOPE (dioleoylphosphatidylethanolamine)	Synthetic polymer poly-(ethylene glycol) (PEG). E.g. PEG1900-lipid, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-PEG_{2000} ($DSPE\text{-}PEG_{2000}$)
3.	Pharmacodynamic Properties (Basic mechanism of action)	Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases.	The stealth liposomes are constructed with PEG (Polyethylene Glycol) studding the outside of the membrane. The PEG coating, which is inert in the body, allows for longer circulatory life for the drug delivery mechanism.
4.	Pharmacokinetic parameters	Conventional liposomes have comparatively lowered half lives and due to the phagocytic reactions, these are considered dose dependent pharmacokinetics.	The dosage-independence of the pharmacokinetics of stealth liposomes and their lack of MPS saturation within the therapeutic dose range are two more assets, in addition to the prolonged circulation half-lives.

Hence liposomes are understood as an important model system in several different basic sciences and as a viable alternative in several applications. The implementation of liposomes in anticancer and possibly other chemotherapies, gene therapy as well as some other medical applications such as artificial blood will serve as an important clinical indication in delaying the basic pharmacokinetic properties. There basic clinical requisites are of immense periphery in therapeutic and clinical diagnostics.

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Article History:----------
Date of Submission: 22-09-10
Date of Acceptance: 06-12-10
Conflict of Interest: NIL
Source of Support: NONE