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Lipases, liposomes and lipid-prodrugs

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ABSTRACT

Colloidal and interfacial phenomena lie at the core of drug formulation, drug delivery, as well as drug binding and action at diseased sites, e.g., in cancer therapy. We review a class of liposome-based drug-delivery systems whose design and functional properties are intimately controlled by the stability of sub-micron structures, lipid-bilayer interfaces, and interfacially activated enzymes that can be exploited to target and deliver drugs. Moreover these drugs can themselves be special lipid molecules in the form of lipid prodrugs that both form the liposomal carrier as well as the substrate for endogenously upregulated lipases that turn the prodrugs into potent drugs precisely at the diseased site.

1. Introduction

The introduction of new drugs is severely hampered by a lack of understanding of the complexity in tailoring the formulation of the drug to overcoming the various biological barriers in the body (see e.g., [1,2]) Many very promising and potent drugs never make it to the clinic because the formulations fail and the drug only makes it to the target in too low concentrations to render it effective. Whereas many drug entities are designed more or less rationally by medicinal chemical approaches to e.g., target specific receptors or DNA binding sites, it is less common that the drug and an appropriate delivery system are designed as a joint entity. Moreover, many drugs, after having been designed, must be modified chemically to enhance their pharmacokinetics and stability as well as to furnish effective targeting and to be able to bypass the immune defense system and overcome the various biological barriers on the drugs' way to the target. Many of the problems involved in dealing with these difficulties are of a nature where colloidal and interfacial effects play a significant role.

Ever since its discovery by Alec Bangham in the early 1960s [3], the liposome has been seen as a unique carrier of drugs because it can be made of biocompatible materials, it is a soft material, it is bottom-up self-organized and self-assembled, versatile, diverse, plastic, adaptable, flexible, fluid, length-scale tunable, durable, as well as to some extent self-repairing and self-healing [2,4–7]. In particular, the liposome can be produced as a unilamellar vesicle small enough, e.g., 100 nm, to allow it to venture into the finest capillaries. The problem of obtaining long circulation times and effectively evading the innate immune system was solved by the stealth technology in the early 1990s, thereby opening up for both passive and active targeting strategies [4,8]. However, despite its many obvious virtues, the liposome has only made it to the clinic in the form of products in very few cases, particularly for cancer therapy. The liposomal formulations authorized for cancer treatment are PEGylated liposomal doxorubicin (Doxil, Lipo-Dox), non-PEGylated liposomal doxorubicin (Myocet) and daunorubicin (DaunoXome), and multilamellar liposomal cytarabine (DepoCyt) [9]. One of the main problems has been to effectively release the encapsulated drug at the target cells and open up efficient transport routes into the cells.

In this topical paper we will review and describe recent advances in the use of a particular kind of liposomes, the so-called LiPlasomes [10], that have been developed to effectively encapsulate anticancer drugs and to take advantage of the endogenous upregulation of certain lipases, *viz* secretory phospholipase A_2 ($sPLA_2$), as a targeting and release mechanism. We will describe and evaluate the most common platforms for liposomal anticancer drug delivery; namely conventional, stealth, active-targeted, and bio-responsive liposomes. We will also elaborate on a range of model systems that have been used to elucidate the critical bottlenecks in forming and stabilizing such liposomes, loading the liposomes, releasing the drugs by various types of lipases, designing lipid prodrugs (and double-lipid prodrugs), formation of drugs by lipase action on the prodrugs, and the use of lipase hydrolysis products for enhancing adsorption and permeation

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at the target cells. At many stages in the route from liposomal formulation to the actual delivery, aspects of colloidal stability, inter-layer forces, interfacial structure, and interfacially activated enzymes, as well as destabilization of the lipid bilayer of the target cell come into play.

2. Platforms for tumor-targeted liposome drug delivery

Liposomes have gained extensive attention as drug delivery vehicles, particularly in antitumor therapy, since their potential was underlined by Gregoriadis and his co-workers in the beginning of the 1970s [11]. However, early liposomal formulations suffered from several drawbacks in relation to lack of selective targeting as well as poor *in vivo* performance and tolerability. For instance, the mononuclear phagocyte system (MPS) [12], nonspecific binding of selective serum proteins (opsonisation) [13], and the complement system [14,15] all decrease the circulation time of conventional liposomes in the blood stream. In addition, interactions with lipoprotein particles in the blood serum were also reported to have a destabilizing effect on liposomes leading to premature drug release [16]. In recent years, lipid-based drug-delivery systems have been considerably improved due to advancements in lipidology and surface modification of liposomes as well as to a better understanding of the pharmacokinetics of colloidal particles. Moreover, advancements in organic chemical synthesis have lead to the realization of more complex molecules, which may prove useful for future liposomal drug delivery systems. One interesting example is vesicles made of amphipathic vase-shaped cavitands [17,18]. The new reported vesicles will add another facet to the field, due to their ability to additionally host small molecules in the cavities (cavitands) on the surface of the vesicles. In the following Sections (2.1 – 2.3), the main strategies for development of liposomes for antitumor therapy, which are depicted in a schematic illustration in Fig. 1, will be briefly discussed.

2.1. Polymer-stabilized long circulating liposomes

The key to optimizing the pharmacokinetic profile of liposomes is to control both the size and physicochemical properties of the liposomes. In general, it has been shown that small unilamellar vesicles (SUVs) in *in vivo* experiments are less liable to be subjected to opsonisation and MPS uptake in the liver compared to multilamellar vesicles (MLVs) [15]. Addition of cholesterol to the lipid mixture enhances serum stability of liposomes [16]. Likewise, usage of lipid compositions with a high phase transition temperature enhances liposome stability *in vivo*, because of a better lipid chain packing [19]. Nevertheless, the most pivotal development to increase liposome stability is flexible biocompatible polymers attached to the liposomal surface, creating so-called stealth liposomes [20–22]. Of the polymers used in formulations at present, polyethylene glycol (PEG) is the most predominant due to the polymer biocompatibility [23], low cytotoxicity [24], and good excretion kinetics [25]. Besides creating a spatial barrier that ensures a strong inter-bilayer repulsion, which increases liposome stability [26], the polymers prevent the binding of opsonins to the liposomal surface, thus shielding the liposomes from MPS recognition. This in turn prolongs the circulation time enabling a better bio-distribution of the liposomes [8]. Application of long-circulation liposomes can enhance the accumulation of liposomes in certain forms of cancer, which have poor lymphatic drainage and discontinuous endothelial lining in the tumor vasculature, which is known as the enhanced permeability and retention (EPR) effect [27]. Nowadays, there are also several modified PEG constructs with different functional groups at the distal end of the polymer, which allow the covalent attachment of targeting moieties [6]. One recent and interesting approach is the introduction of chemical linkages between the PEG polymer and the lipid anchor that enables the removal of the PEG polymer under specific local conditions such as lowered pH in the tumor tissue or in the endosomal environment. Examples of such linkages are dithiobenzylurethane [28], the hydrazone bond [29], and the α-phenyl substituted vinyl ether (PIVE) bond [30].

2.2. Active targeting of liposomes towards cancer biomarkers

While passive targeting with long-circulation liposomes is beneficial to enhance local accumulation of liposomes, active targeting towards specific cancer biomarkers is needed for selective delivery and efficient drug internalization. Several types of cancer overexpress various membrane receptors, which can be targeted by covalently attaching targeting units to the liposomal surface. The choice of the targeting moiety is largely influenced by the biochemical characteristics of the cancer type as well as by whether the liposome payload should be released extra- or intracellularly. A schematic illustration demonstrating several types of ligands used in active targeting is shown in Fig. 2. Immunoglobulins, particularly IgG, as a whole or a fragment, have been thoroughly investigated as targeting units for liposomes (immunoliposomes) [31]. For instance, antibodies towards Human Epidermal Growth Factor Receptor 2 (HER2) coupled to liposomes have been tested *in vivo* and found to exhibit a large increase of delivered doxorubicin-loaded liposomes to HER2-overexpressing cells [32]. Though immunoliposomes demonstrate high specificity and much is known about antibody biochemistry, certain drawbacks have been observed. Antibodies have been shown to invoke immunogenic response upon administration [33,34]. Also, their affinity towards the antigen of interest can be impaired by the approach used to immobilize the antibody on the liposome surface [35]. Another protein used in targeting is transferrin, an 80 kDa iron binding serum

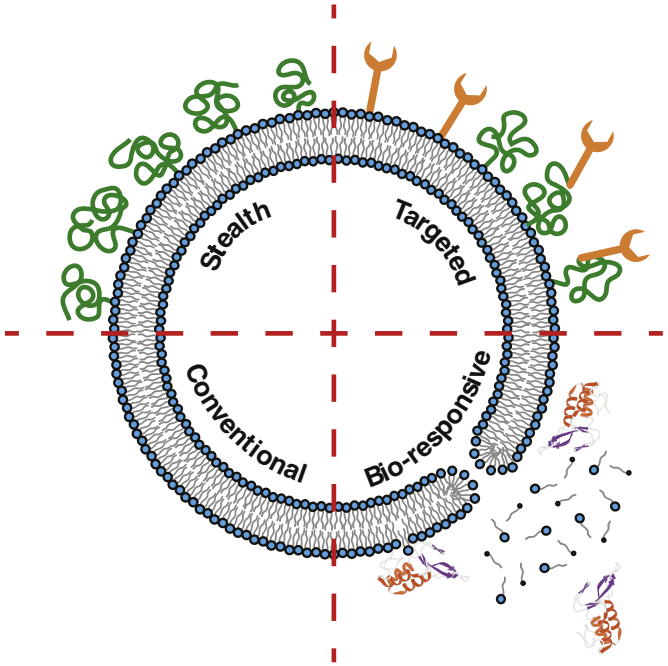

Fig. 1. Schematic overview of the major classes of liposomal platforms for anticancer drug delivery. Conventional liposomes are composed of plain phospholipids. Stealth liposomes have lipophilic polymers attached to their exterior leaflet, which will improve their pharmacokinetic properties. Active targeted liposomes have ligands towards cancer biomarkers covalently attached to either the liposomal surface or the distal ends of hydrophilic polymers. Bio-responsive liposomes have their lipid composition engineered to respond to the tumor-specific conditions or to external stimuli. For instance, lipase-labile liposomes will be enzymatically degraded aiding the release of the drug at the target and possibly enhancing drug permeation into the target cells. The phospholipase A_2 structure was obtained from [pdb: 1POC] [178].