

Active and Tumour Specific Drug Release Using Targeted Liposomal Drug Carriers

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Introduction

The search for new and better anticancer drugs for the growing number of cancer patients remains one of modern medicine's greatest quests. Although several novel anticancer drugs have recently been developed that show promising effects in cell culture studies, clinical use is often prohibited due to non-targeted, inadequate delivery of therapeutic concentrations to the cancer tissue at doses causing severe toxic effects on normal organs. Liposomal drug delivery systems serve as prospective targeted microcarriers of anticancer drugs to cancer tissue. However, the first generation of marketed drug delivery systems has shown limited therapeutic advantages due to an insufficient tumour specific delivery and release of the liposomal payload. A rational development of novel liposomal drug delivery principles that can lead to improved tumour specific drug delivery and release is needed.

Liposomal Drug Carriers - Potentials and Drawbacks

Liposomes were suggested as drug carriers in cancer therapy over 30 years ago (Gregoriadis *et al.*, 1974). Since then, the interest in using liposomes for tissue specific transport of drugs has steadily grown and liposomal drug delivery systems are now being studied as promising drug carriers (Bangham, 1995; Gregoriadis, 1995; Drummond *et al.*, 1999). Initially, the use of liposomal drug delivery systems suffered from very fast blood clearance by the reticuloendothelial system (RES) (Drummond *et al.*, 1999) and liposomes were first recognised as prospective drug delivery systems when it was discovered that incorporation

of negatively charged gangliosides (GM₁) or polymer (PEG) linked lipids resulted in a significant increase in blood circulation time (Gabizon and Papahadjopoulos, 1988). Although liposomes in this way are effective carriers of high concentrations of encapsulated drugs to cancer tissue, it is now clear that sufficient local drug bioavailability after accumulation in the cancer tissue is critical (Barenholz, 2001; Allen and Cullis, 2004).

Current clinically approved liposomal Stealth formulations such as Doxil[®] have only resulted in a modest increase in antitumour activity (Harrington *et al.*, 2001; Judson *et al.*, 2001; Thomas *et al.*, 2001; Rimassa *et al.*, 2003). One reason is the lack of an effective and active release mechanism in cancer tissue. The release of encapsulated doxorubicin from Doxil[®] after accumulation in cancer tissue is poorly understood, but is likely to involve non-specific chemical disruption of the pH-loading gradient retaining the drug inside the liposome (Barenholz, 2001). When this loading gradient is partly lost without any prior degradation of the liposomal carrier, a slow drug release will take place. The long circulation profile of Doxil liposomes combined with the lack of an active trigger mechanism has furthermore introduced new dose limiting side-effects (Gabizon, 2001; Charrois and Allen, 2003). In addition, the mechanism by which doxorubicin is released from Stealth liposomes in the cancer tissue may not apply to other classes of passively encapsulated drugs, which cannot easily diffuse across the intact liposomal membrane. In fact, the absence of an active trigger and the use of a passive loading method provides an explanation for the lack of antitumour activity and toxicity of cisplatin containing Stealth liposomes (SPI-077), although it was

found that these accumulated substantially in the tumour tissue (Bandak *et al.*, 1999; Kim *et al.*, 2001; Vail *et al.*, 2002).

It is of the utmost importance to develop new liposomal drug delivery systems that are tailored to accumulate and release the encapsulated drugs in the tumour tissue if liposomes are to fulfil their potential as superior targeted microcarriers (Allen and Cullis, 2004). Optimally, the liposomal drug release rate should be adjusted in a way that matches the liposomal residence time in the cancer tissue and the pharmacodynamic profile of the carried drug (Barenholz 2001; Andresen *et al.*, 2004a). There are two general directions within liposomal drug delivery research focusing on accomplishing these tasks: site specific delivery by targeting and site specific triggering. In the following sections, some of the recent and prospective methods in site specific targeting and triggering will be presented with particular focus on new strategies involving cancer selective enzymes as tumour specific triggers.

Active Liposomal Targeting and Release Strategies

Enhanced tumour accumulation of liposomes via active targeting

Liposomes with ligands attached to the surface are potential candidates for delivery of the encapsulated drug to specific target sites. Some of the methods developed to achieve active targeting include liposomes coupled with specific antibodies as well as liposomes coated with ligands targeting proteins overexpressed on cancer cells or endothelial cells lining the newly formed blood vessels in the tumour (Sapra and Allen, 2003; Gabizon *et al.*, 2004). Examples of specific proteins that are expressed on the surface of actively growing tumour cells are the folate receptor (Aronov *et al.*, 2003) and the integrin receptor (Hood *et al.*, 2002). Another example includes galactolipids that increase transfection by targeting the asialoglycoprotein receptor (Maruyama *et al.*, 1999).

Antibody coated liposomes (immunoliposomes) have been extensively studied, either with the antibodies attached directly to the phospholipid headgroup or to the terminus of the PEG polymer. The latter approach has been most successful due to better accessibility of the antibodies to the target proteins on the cancer cell surface (Maruyama *et al.*, 1999). One of the major challenges involved in coating liposomes with antibodies directed against tumour targets, is to tune the fine balance between having sufficient antibodies attached to the liposome to achieve a targeted binding and retention in the tumour and to avoid the enhanced RES clearance in the blood stream that increases with the number of antibodies attached to the surface of the liposomal carrier (Maruyama *et al.*, 1999; Gabizon *et al.*, 2004).

Although the liposomal targeting concept using specific ligands attached to the surface of the liposomal carrier can potentially lead to an increase in antitumour efficacy and

a decrease in toxicity, further preclinical and clinical studies are still required to demonstrate the validity of this concept (Gabizon *et al.*, 2004).

Enhanced tumour release from liposomes via active triggering

Several strategies have been proposed to achieve a triggered drug release once the liposomes accumulate in the cancer tissue. Liposomes triggered by changes in pH (Litzinger and Huang, 1992; Reddy and Low, 2000), temperature (Kong *et al.*, 2000) and light (Spratt *et al.*, 2003) have been tested and show promising effects as site specific triggers of encapsulated drugs in cancer tissue. However, liposomes designed with these specific trigger mechanisms have not yet reached the pharmaceutical market. The light and temperature triggered concept that is based on exogenously applied stimuli furthermore requires precise localisation of the primary tumour and is not suitable for treatment of undetected metastases. A more recently proposed and viable principle for tumour specific drug release is the enzymatically triggered approach relying on a key difference in enzyme expression between healthy and diseased tissue.

Tumour upregulated enzymes as liposomal triggers

The use of enzymes, that are upregulated in tumour tissue, is probably the most intriguing endogenous trigger principle. Strategies involving the design of specific lipid conjugates that are activated by tumour-expressed enzymes resulting in the generation of fusogenic liposomes in the cancer tissue have been suggested. Examples of enzymatic triggers that have shown promising effect in preclinical studies include tumour-associated proteases and phosphatases that are possible candidates for enzymatically triggered liposomal fusion leading to intracellular drug transport and release (Davis and Szoka, 1998; Meers, 2001). The use of sphingomyelinase and phospholipase C as enzymatic triggers have also been investigated (Goni and Alonso, 2000; Villar *et al.*, 2001).

Secretory phospholipase A2 as a tumour specific trigger

Secretory phospholipase A2 (sPLA2) is a small interfacially active enzyme that catalyses the hydrolysis of phospholipids producing free fatty acids and lysolipids. sPLA2 is highly overexpressed in inflammatory and cancer tissue (Abe *et al.*, 1997; Jiang *et al.*, 2002; Laye and Gill, 2004). Hence, sPLA2 is an obvious and ideal tumour specific trigger enzyme that can be used to puncture liposomes that are engineered to be degradable by sPLA2 once they accumulate in the cancer tissue (Davidsen *et al.*, 2003; Jørgensen *et al.*, 2004). Furthermore, sPLA2 can work as a tumour specific activator of a novel class of prodrug lipids that are suitable as building blocks for a new class of prodrug liposomes as schematically shown in Figure 1 (Andresen *et al.*, 2004b; Jensen *et al.*, 2004). In addition, this new and integrated prodrug and drug delivery system

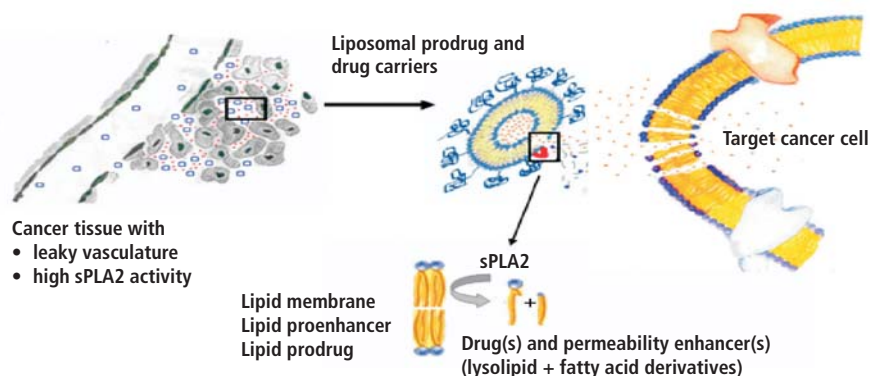


Figure 1 – Overview of the sPLA2 triggered liposomal prodrug and drug delivery concept. Polymer covered prodrug liposomes are stable in the bloodstream and accumulate in the porous cancer tissue with high levels of sPLA2. In this way the sPLA2 triggered release and activation of the lipid prodrugs and the encapsulated drugs becomes site specific to the malignant target tissue (Jensen et al., 2004).

can advantageously be used to encapsulate and transport conventional anticancer drugs to the cancer tissue (Andresen et al., 2004a).

A particular beneficial feature of using sPLA2 as a trigger is the observation that the enzyme's activity is much higher towards aggregated substrates such as liposomes compared with lipid monomers. Furthermore, sPLA2 is secreted into the extracellular space of the cancer tissue where the liposomes tend to accumulate after extravasation (Drummond et al., 1999). In addition, the degradability of the liposomes by sPLA2 can be rationally optimised by changing the lipid composition and the biomaterial properties of the liposomal membrane (Mouritsen and Jørgensen, 1998; Davidsen et al., 2003). Finally, it has been shown that the steric barrier induced by the polymer coverage does not prevent sPLA2 from reaching the liposomal surface, where the interfacial

lipid hydrolysis occurs (Andresen et al., 2004a). This is in accordance with other surface interaction studies showing that polymer grafted liposomes are prone to opsonisation and protein absorption (Moghimi and Szebeni, 2003).

An example of the growth inhibition of cancer cells caused by sPLA2 is given in Figure 2. Pronounced growth inhibition was observed when the empty prodrug liposomes were added to the gastric human cancer cells secreting high amounts of sPLA2. External addition of sPLA2 resulted only in a marginal decrease in cell growth indicating complete and fast hydrolysis of the prodrug lipids by sPLA2. When a specific sPLA2 inhibitor was co-incubated with the prodrug liposomes, the cell growth was almost restored proving the non-toxic properties of the prodrug lipids and demonstrating the ability of sPLA2 to convert the inactive prodrug lipids to active cytotoxic anticancer lipids.

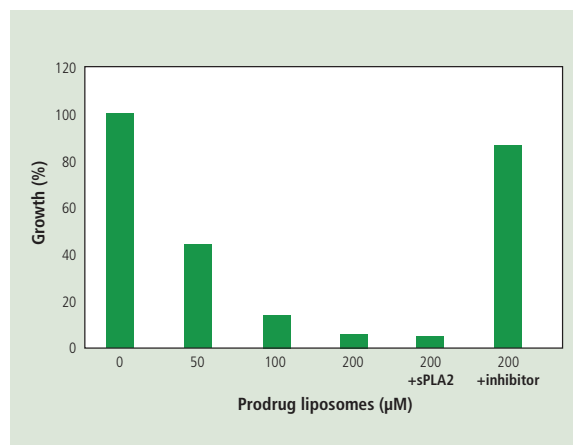


Figure 2 – Concentration dependent cytotoxicity of prodrug liposomes activated by sPLA2 on KATO III gastric cancer cells. The marginal cytotoxicity observed after addition of the specific sPLA2 inhibitor proves the ability of sPLA2 secreted by the cancer cells to activate the non-toxic prodrug lipids to effective anticancer lipids. External addition of sPLA2 resulted only in a marginal increase in cytotoxicity demonstrating efficient prodrug hydrolysis by sPLA2 secreted by the cancer cells (Andresen et al., 2004a).

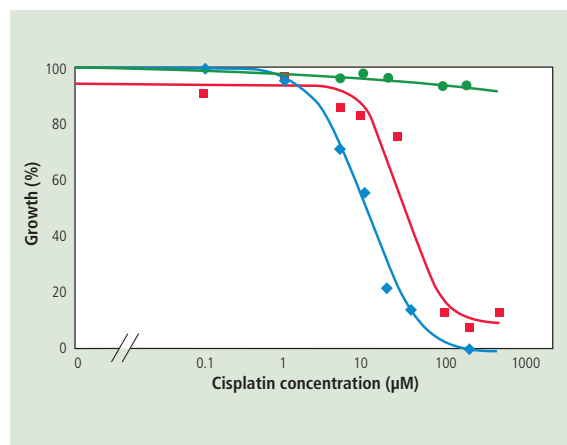


Figure 3 – Growth of HT-29 colon cancer cells after exposure to free cisplatin (red), cisplatin encapsulated in sPLA2 degradable liposomes (blue) and SPI-077 Stealth liposomes (green). After 24 hours' incubation, fresh media was added, and the number of cells was evaluated after 3 days.

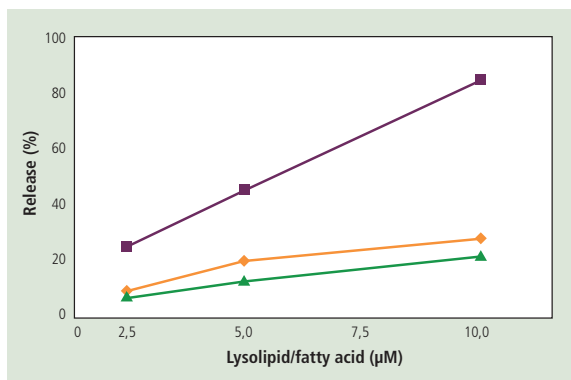


Figure 4 – Permeability enhancing effects of sPLA2 generated hydrolysis products. Concentration dependent release of the encapsulated model drug, calcein, from liposomes 20 min after addition of lysolipids (▲), free fatty acids (◆), and equimolar mixtures of the lysolipids and free fatty acids (■) (Davidsen et al., 2002).

The sPLA2 trigger principle can readily be extended to release various anticancer drugs that are encapsulated into the sPLA2 degradable liposomes. Figure 3 shows an example of the triggered release of encapsulated cisplatin from sPLA2 degradable liposomes. Significant cytotoxic activity was observed when the sPLA2 degradable liposomes with encapsulated cisplatin were added to colon cancer cells. In contrast, the clinically used liposomal Stealth formulation with encapsulated cisplatin, (SPI-077), showed an insignificant cytotoxicity in accordance with earlier reports (Bandak et al., 1999). It should also be noted that the sPLA2 degradable liposomes loaded with cisplatin were significantly more active than free cisplatin possibly due to an additive cytotoxic membrane perturbing effect of the hydrolysis products. This cell membrane perturbing effect might also facilitate the transmembrane diffusion of cisplatin into intracellular target sites. The ability of the generated lysolipid and fatty acid hydrolysis products to increase transmembrane diffusion has been investigated in model lipid membranes studies (Davidsen et al., 2002). As shown in Figure 4, a remarkable synergistic membrane permeabilising effect was observed supporting the use of sPLA2 as a dual trigger of enhanced liposomal drug release and enhanced intracellular drug transport. Interestingly, it has recently been shown that a particular anticancer lysolipid, (hexadecylphosphocholine, HPC) functions as an effective gene transfer enhancer *in vivo* possibly as a result of its membrane permeability enhancing properties (Settelen et al., 2004).

Future Prospects

The sPLA2 trigger mechanism may prove useful in the development of several novel anticancer lipid based prodrugs, which can be used to make liposomal drug carriers that selectively target the tumour and undergo a site-specific triggered activation and release. Anticancer agents such as docosahexanoic acid (DHA) (Siddiqui et al., 2001), retinoic acids (Altucci and Gronemeyer, 2001) or prostaglandins (Straus and Glass, 2001) can readily be ester-linked to prodrug lipids to create a double-prodrug liposomal delivery system, which would allow for tumour specific transport and release of anticancer lipids, retinoids, DHA or prostaglandins. In addition, the sPLA2 degradable prodrug liposomes are applicable for combined transport and tumour specific delivery and release of encapsulated compounds such as radiation sensitisers, cytokines, immunomodulators or conventional therapeutics (Harrington et al., 2002). In all these cases, targeting using sPLA2 as an active tumour specific trigger to convert the non-toxic lipid prodrugs to effective anticancer drugs and release of the encapsulated drugs specifically in the cancer tissue is likely to decrease toxicity and increase antitumour efficacy resulting in an improvement of the therapeutic index.

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