

Secretory Phospholipase A₂ Responsive Liposomes

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ABSTRACT: Secretory phospholipase A₂ (sPLA₂) expression is increased in several cancers and has been shown to trigger release from some lipid carriers. This study used electrospray ionization mass spectrometry (ESI-MS) and release of 6-carboxyfluorescein (6-CF) to determine the effects of sPLA₂ on various liposome formulations. Different combinations of zwitterionic [1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine, and 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE)] and anionic [1,2-distearoyl-*sn*-glycero-3-phosphatidic acid, 1,2-distearoyl-*sn*-glycero-3-phosphatidylglycerol (DSPG), 1,2-distearoyl-*sn*-glycero-3-phosphatidylserine, and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-poly(ethylene glycol) 2000 (DSPE-PEG)] phospholipids were examined. DSPG and DSPE were most susceptible to sPLA₂-mediated degradation compared with other phospholipids. Increased 6-CF release was observed after inclusion of 10 mol % DSPE and anionic lipids into different liposome formulations. Group IIa sPLA₂-mediated 6-CF release was less than Group III and relatively insensitive to cholesterol (Chol), whereas Chol reduced sPLA₂-mediated release. Inclusion of DSPE-PEG increased sPLA₂-mediated 6-CF release, whereas serum reduced lipid degradation and 6-CF release significantly. These data demonstrate that ESI-MS and 6-CF release were useful in determining the selectivity of sPLA₂ and release from liposomes, that differences in the activity of different sPLA₂ isoforms exist, and that DSPE-PEG enhanced sPLA₂-mediated release of liposomal constituents. These findings will aid in the selection of lipids and optimization of the kinetics of drug release for the treatment of cancers and diseases of inflammation in which sPLA₂ expression is increased. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association *J Pharm Sci* 100:3146–3159, 2011

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INTRODUCTION

Lipid based nanoparticulate drug carriers, such as long-circulating sterically-stabilized liposomes (SSL),

Abbreviations used: 6-CF, 6-carboxyfluorescein; Chol, cholesterol; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DSPA, 1,2-distearoyl-*sn*-glycero-3-phosphatidic acid; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine; D₇₀-DSPC, 1,2-distearoyl(deuterated 70)-*sn*-glycero-3-phosphatidylcholine; DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine; DSPE-PEG, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol) 2000]; DSPG, 1,2-distearoyl-*sn*-glycero-3-phosphatidylglycerol; DSPS, 1,2-distearoyl-*sn*-glycero-3-phosphatidylserine; ESI-MS, electrospray ionization mass spectrometry; FA, fatty acid; FBS, fetal bovine serum; LPC, lysophospholipid; PEG, polyethylene glycol; PLA₂, phospholipase A₂; SPRL, secretory phospholipase A₂ responsive liposomes; SSL, sterically-stabilized liposome; sPLA₂, secretory phospholipase A₂.

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can encapsulate drugs stably, alter their pharmacokinetics radically compared with free drug, and confer new pharmacological activity.^{1–3} Differences in drug carrier circulation half-life and tissue/tumor biodistribution are believed responsible for their improved antitumor activity and reduced toxicity.^{4,5} The clinical advantage of nanoparticles, such as SSL, was established with the approval of Doxil® (Centocor Ortho Biotech, Horsham, PA) and other liposome formulations in the USA. Following administration, SSL can accumulate passively in solid tumors due to the enhanced permeability and retention effect mediated by defects in the vasculature and lack of functional lymphatics.^{6,7} Despite increased accumulation of SSL into tumors, for many drugs the rate of drug release is not optimal and clinical utility is limited.⁸ To overcome these barriers, a variety of physical and physiological approaches are being examined to facilitate and control drug release; these include exposure to light,^{9–11} heat,^{12–14} and use of ultrasound.^{15,16}

In this study, we choose to exploit pathophysiological differences in enzyme expression in normal and malignant tissues, that is, differences in secretory phospholipase A₂ (sPLA₂) expression,^{17–19} to modulate drug release. Previous studies examined the effect of porcine pancreatic- and bee venom sPLA₂ on phosphatidylcholine vesicles with different physical attributes.²⁰ These studies suggested that sPLA₂ present in some pathologies stimulate drug release from lipid-based drug carriers, such as liposomes (Fig. 1).^{21–23} In contrast, reduced expression of sPLA₂ in nondiseased tissues would limit lipid degradation and drug release, and is hypothesized to reduce toxicity (Fig. 1).

Recent studies demonstrated that sPLA₂ expression and activity is increased in prostate,^{24–27} breast,^{28–30} and pancreatic^{31–33} cancers. In prostate cancers, Group IIa sPLA₂ is reported to be expressed at levels 22-fold greater than disease-free paired controls.^{26,27,34} Increased sPLA₂ expression in cancer tissues correlates to increased immunohistochemical staining at the plasma membrane.²⁶ This coincides with the proposed mechanism of sPLA₂ action and suggests that these increases are localized to the site of injury. Typically, there is limited sPLA₂ in the systemic circulation, except in the case of septic shock or inflammation.

Secretory phospholipase A₂ are esterases that cleave glycerophospholipids, such as phosphatidyl-

choline, at the *sn*-2 ester bond, releasing a fatty acid (FA) and a lysophospholipid (LPC).³⁵ Many investigators have examined the effect of sPLA₂ on cellular membranes, lipid vesicles, and lipid-based drug carriers, but a majority of these studies were limited to bee venom (Group III), snake venom (Group Ia) or porcine pancreas (Group Ib) sPLA₂.^{20,36,37} In contrast, there are few published studies that examined the effect of human sPLA₂ on the degradation of lipid-based drug carriers²³ or made comparisons between the different isoforms.

The overall goal of this research was to determine the effect of sPLA₂ on lipid profiles and on the rate and extent of drug release from lipid nanoparticulate drug carriers. This was accomplished by examining the effect of different sPLA₂ on individual and mixed lipid degradation using electrospray ionization mass spectrometry (ESI-MS). This information was then used to formulate prototype sPLA₂ responsive liposomes (SPRL). The functional activity of sPLA₂ was determined by assessing the release of 6-carboxyfluorescein (6-CF), an aqueous soluble fluorescent marker encapsulated in conventional SSL and SPRL formulations in buffer and serum. Understanding the time course of sPLA₂-mediated lipid degradation and 6-CF release will accelerate the rational development of drug carriers to achieve optimal drug exposure selectively, thus enhancing drug efficacy and minimizing nontarget tissue toxicity.

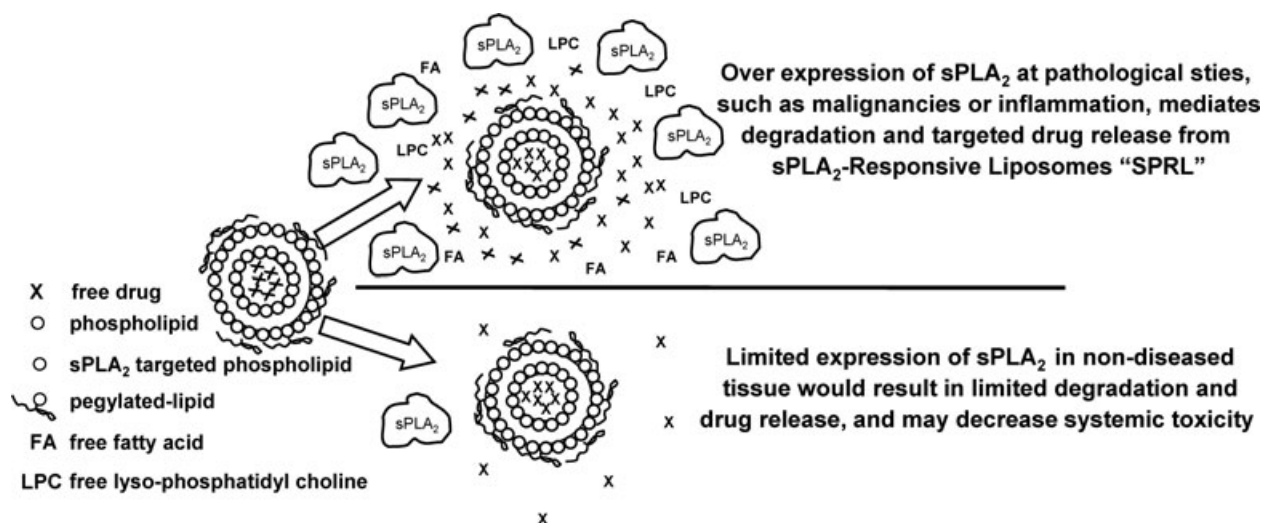


Figure 1. sPLA₂-facilitated drug release. This illustration describes the proposed mechanism of sPLA₂-mediated degradation and drug release from liposomes. Increased expression of sPLA₂ in solid tumors or other diseased tissue would enhance the degradation of phospholipids resulting in increased membrane permeability and drug release. Increased lipid degradation would be evidenced by increases in fatty acid and lysophospholipid lipid levels. Limited expression of sPLA₂ in noncancerous or diseased tissues would result in reduced lipid degradation and drug release.

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