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A novel class of photo-triggerable liposomes containing DPPC:$DC_{8,9}PC$ as vehicles for delivery of doxorubcin to cells

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ABSTRACT

Success of nanoparticle-mediated drug delivery is subject to development of optimal drug release strategies within defined space and time (triggered release). Recently, we reported a novel class of photo-triggerable liposomes prepared from dipalmitoyl phosphatidylcholine (DPPC) and photopolymerizable diacetylene phospholipid ($DC_{8,9}PC$), that efficiently released entrapped calcein (a water soluble fluorescent dye) upon UV (254 nm) treatment. To develop these formulations for *in vivo* applications, we have examined phototriggering of these liposomes by visible light, and the effect of released anticancer drugs on cellular toxicity. Sonicated liposomes containing various ratios of DPPC:$DC_{8,9}PC$ and 4 mol% DSPE-PEG2000 were loaded with calcein (Ex/Em, 485/517 nm) or a chemotherapy drug, Doxorubicin (DOX, Ex/Em 490/590 nm). Our initial experiments showed that 514 nm laser treatment of liposomes containing 10 or 20 mol% $DC_{8,9}PC$ for 1–3 min resulted in significant release of calcein. Based on these results, we performed studies with DOX-loaded liposomes. First, biophysical properties (including liposome size and stability) and DOX encapsulation efficiency of the liposomes were determined. Subsequently, the effect of 514 nm laser on DOX release, and cellular toxicity by released DOX were examined. Since liposomes using the 86:10:04 mole ratio of DPPC:$DC_{8,9}PC$:DSPE-PEG2000, showed highest encapsulation of DOX, these formulations were investigated further. We report that (i) liposomes retained about 70% of entrapped DOX at 37 °C in the presence of 0–50% serum. (ii) 514 nm laser treatment resulted in DOX release from liposomes in a wavelength-specific manner. (iii) Laser treatment of co-cultures containing DOX-loaded liposomes and cells (Raji and MCF-7) resulted in at least 2–3 fold improved cell killing as compared to untreated samples. Taken together, the photo-triggerable liposomes described here may provide a platform for future drug delivery applications. To our knowledge, this is the first report demonstrating improved cell killing following light-triggered release of an encapsulated anticancer agent from photosensitive liposomes.

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1. Introduction

Several nano-delivery systems that are amenable to release drugs at desired sites have been developed since decades. Among these, intensive research has been conducted using the liposomes as triggerable drug carriers [1]. Various triggering modalities examined to date include local hyperthermia, use of metal ions, pH, enzymes and light (radiation) [2–4].

Electromagnetic radiation-sensitive liposomes present a promising system and rely on strategically designed phospholipid molecules to initiate light-induced trigger [5]. The principle(s) of phototriggering include photopolymerization of lipids [6], photosensitization by membrane anchored hydrophobic probes [7–10], or photoisomerization of photoreactive lipids [11]. However, none of the formulations developed so far have been successful for *in vivo* applications presumably due to the lack of adequate photon energy produced by the radiation source(s) or inability of radiation sources used to penetrate into biological tissues [5]. Therefore, alternate methods to develop liposomes sensitive to tissue-penetrating wavelengths are warranted for their successful applications in the clinic.

Photo-triggerable liposome formulations containing wavelength-specific photosensitizers (primarily lipidic and/or hydrophobic in nature) in conjunction with photoactivable lipids are also described. For example, inclusion of the cationic dye, 1,1′-didodecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), induces destabilization of DOPE:SorbPC (3:1) liposomal membranes when radiated at 550 nm [12]. In another example, researchers have studied light-sensitive liposomes based on photochemical triggering, such as plasmenylcholine activation by a near infra red (NIR) sensitizer. Plasmenylcholine

Abbreviations: *DPPC*, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; *Egg PC*, L-α-Phosphatidylcholine (egg, chicken); *$DC_{8,9}PC$*, (1,2 bis (tricosa-10 12-diynoyl)-*sn*-glycero-3-phosphocholine); *DSPE-PEG2000 (18:0 PEG2 PE)*, 1,2-Distearoyl-*sn*-Glycero-3 Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (ammonium salt); *HEPES buffer (HBS)*, 10 mM HEPES, 140 mM NaCl (pH 7.5); *PBS*, 2.66 mM KCl, 1.47 mM KH_2PO_4, 138 mM NaCl, 8.06 mM Na_2HPO_4-$7H_2O$ (pH 7.1)

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photooxidation was initiated by different hydrophobic molecules such as Zn-phthalocyanine, octabutoxyphthalocyanine, and bacterio-chlorophyll-*a*. These sensitizing agents absorb between 630 and 820 nm inducing membrane phase changes that leads to phototriggering of liposomes and release of contents [5]. Although these wavelengths have the advantage of deeper tissue penetration depth for clinical therapeutics, dug release experiments were not established [5,13]. An added asset in developing photo-triggerable nanoparticles is the steady improvements in laser-technology yielding greater ability to control laser systems by the light wavelength, intensity, duration and beam diameter [14].

The photopolymerizable phospholipid, (1,2 bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$) (Fig. 1A), is a lipid present in lower organisms with unique bilayer packing-self-assembly [15]. Due to highly reactive diacetylene groups uniquely assembled in the lipid bilayer, photopolymerization by UV treatment results in chains of covalently linked lipid molecules within the bilayer [16,17]. Diacetylene groups can be photopolymerized by high energy radiation and are used to monitor high energy radiation released in case of a dirty bomb attack [18]. Liposomes prepared from $DC_{8,9}PC$ alone form tube like structures that are not suitable for drug entrapment; therefore, liposome formulations with lipid mixtures were used in our studies (Fig. 1B). Biological applications of $DC_{8,9}PC$ examined thus far include functionalized polymerized vesicles for vascular targeted molecular imaging [19], candidates for oral vaccine preparations [20], and DNA delivery [21,22]. However, *in situ* light-triggered drug release potential of $DC_{8,9}PC$ liposomes has not been reported until recently [23].

Recently, we have demonstrated that liposomes derived from DPPC:$DC_{8,9}PC$ mixture when treated with UV (254 nm) light released their entrapped contents. Since the observed leakage was selective to this lipid mixture, we proposed that the unique packing properties of $DC_{8,9}PC$ in the liposome bilayer were crucial to promote the observed effect (photopolymerization) [6,16]. In this report, we have extended our studies to investigate visible light-triggered drug delivery potential of these liposomes. Our studies show that laser (514 nm) treatment of DPPC:$DC_{8,9}PC$ liposomes results in the release of entrapped calcein (Ex/Em, 485/517 nm) but not calcein blue (Ex/Em 360/460 nm) under identical conditions. This observation suggests that visible light-induced solute leakage from the liposomes shown here depends on the spectral properties of entrapped solutes. Since cellular toxicity by liposomal DOX was also enhanced upon laser treatment, our photo-triggerable liposomes are likely to form the basis for next-generation of clinically suitable nano-drug delivery tools.

2. Materials and methods

2.1. Materials

1,2 bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine, ($DC_{8,9}PC$) was synthesized at Naval Research Laboratory using published literature procedure [24]. All other phospholipids were purchased from Avanti Polar lipids, Inc. (Alabaster, AL, USA). Calcein and calcein blue were purchased from Fluka-Sigma-Aldrich (St. Louis, MO, USA). Agarose beads, Bio-Gel A0.5 m were purchased from Bio-Rad Laboratories, Richmond, CA, USA. Doxorubicin-hydrochloride (DOX-HCl) (Bedford Laboratories, Bedford, OH) was obtained through the NIH Pharmacy, Clinical Center, Bethesda, MD. PD10 columns were from GE Healthcare Lifesciences (Piscataway, NJ). All other reagents and buffers were of reagent grade.

2.2. Cell lines

Lymphoblastoid cells derived from a Burkitt lymphoma (Raji, ATCC CRL-2367) were grown in RPMI media supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U of penicillin/ml, and 100 μg of streptomycin/ml (GIBCO® Media, Invitrogen, USA). Human breast adenocarcinoma cell line (MCF7, ATCC HTB-22) received through the NCI-DCTD Tumor/cell line repository (http://dtp.nci.nih.gov/brances/btb/tumor-catalog.pdf) , were grown in RPMI media with 10% fetal calf 2 mM L-glutamine 100 U of penicillin/ml, and 100 μg of streptomycin/ml (GIBCO® Media, Invitrogen, USA).

2.3. Formation of liposomes

Liposomes were prepared using the probe sonication essentially as described [23]. The following lipid mixtures were used (Table 1):

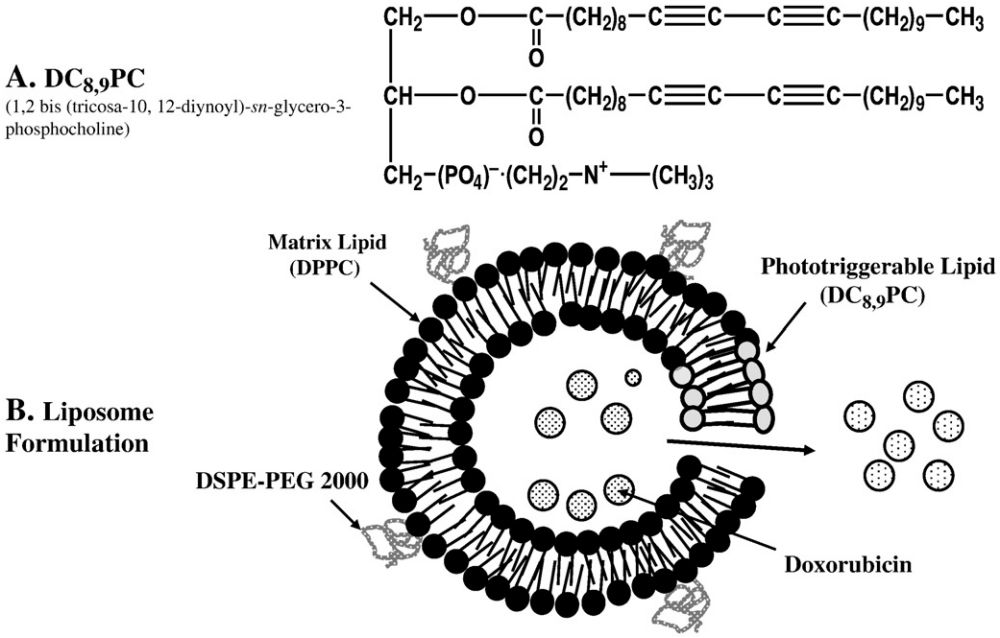

Fig. 1. Design principle of photo-triggerable liposomes. (A) Chemical structure of $DC_{8,9}PC$., (B) A diagram showing various components of DPPC:$DC_{8,9}PC$ liposomes including DPPC (matrix lipid), $DC_{8,9}PC$ (photo-triggerable lipid), DSPE-PEG2000 (stabilizing lipid) and entrapped DOX (anticancer drug). The cartoon also shows light-induced defects in the lipid membrane resulting in release of DOX.