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Distinct solubility and cytotoxicity regimes of paclitaxel-loaded cationic liposomes at low and high drug content revealed by kinetic phase behavior and cancer cell viability studies

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ABSTRACT

Lipid-based particles are used worldwide in clinical trials as carriers of hydrophobic paclitaxel (PTXL) for cancer chemotherapy, albeit with little improvement over the standard-of-care. Improving efficacy requires an understanding of intramembrane interactions between PTXL and lipids to enhance PTXL solubilization and suppress PTXL phase separation into crystals. We studied the solubility of PTXL in cationic liposomes (CLs) composed of positively charged 2,3-dioleyloxypropyltrimethylammonium chloride (DOTAP) and neutral 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) as a function of PTXL membrane content and its relation to efficacy. Time-dependent kinetic phase diagrams were generated from observations of PTXL crystal formation by differential-interference-contrast microscopy. Furthermore, a new synchrotron small-angle x-ray scattering *in situ* methodology applied to DOTAP/DOPC/PTXL membranes condensed with DNA enabled us to detect the incorporation and time-dependent depletion of PTXL from membranes by measurements of variations in the membrane interlayer and DNA interaxial spacings. Our results revealed three regimes with distinct time scales for PTXL membrane solubility: hours for >3 mol% PTXL (low), days for ≈ 3 mol% PTXL (moderate), and ≥20 days for < 3 mol% PTXL (long-term). Cell viability experiments on human cancer cell lines using CL_{PTXL} nanoparticles (NPs) in the distinct CL_{PTXL} solubility regimes reveal an unexpected dependence of efficacy on PTXL content in NPs. Remarkably, formulations with lower PTXL content and thus higher stability show higher efficacy than those formulated at the membrane solubility limit of ≈3 mol% PTXL (which has been the focus of most previous physicochemical studies and clinical trials of PTXL-loaded CLs). Furthermore, an additional high-efficacy regime is seen on occasion for liposome compositions with PTXL ≥9 mol% applied to cells at short time scales (hours) after formation. At longer time scales (days), CL_{PTXL} NPs with ≥3 mol% PTXL lose efficacy while formulations with 1–2 mol% PTXL maintain high efficacy. Our findings underscore the importance of understanding the relationship of the kinetic phase behavior and physicochemical properties of CL_{PTXL} NPs to efficacy.

1. Introduction

The landmark discovery that paclitaxel (PTXL), derived from the Pacific Yew tree, suppresses cell division in tumors [1], has resulted in the ongoing worldwide effort to develop efficient synthetic carriers of PTXL for cancer chemotherapy [2]. PTXL is a hydrophobic molecule (Fig. 1a and b) known to inhibit mitosis by stabilizing microtubules (upon binding a specific hydrophobic pocket on the β-tubulin subunit), thereby obstructing chromosome capture and

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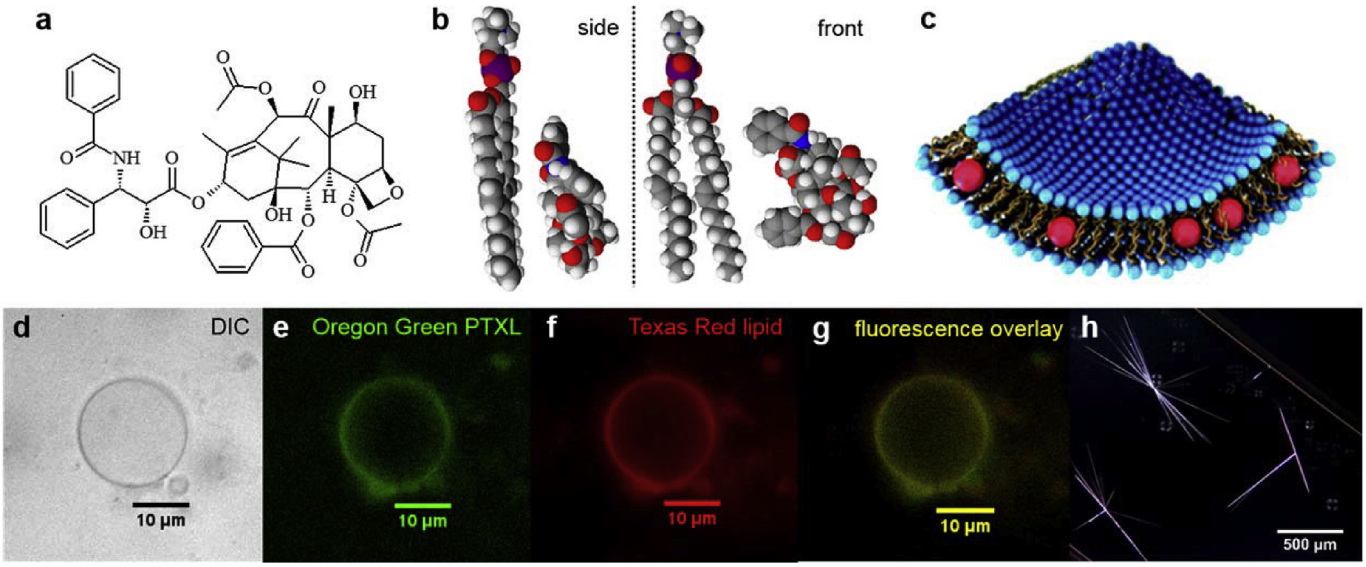

Fig. 1. PTXL-containing liposomes from the molecular to micrometer scale. (a–c) a molecular look at the PTXL–lipid system: a) the chemical structure of PTXL, b) space filling molecular models for DOPC and PTXL viewed from the side and the front, c) schematic drawing of a liposome with hydrophobic molecules (red spheres, representative of PTXL) embedded within the membrane. (d–g) Microscopy images of a singular unsonicated PTXL-containing liposome (composed of a 90:10:5:7.1 mol ratio of DOTAP:DOPC:OregonGreen-PTXL:TexasRed-DHPE), demonstrating colocalization of PTXL with the lipid bilayer: d) differential-interference-contrast image, e) green fluorescence due to Oregon Green-conjugated PTXL, f) red fluorescence from the TexasRed-DHPE lipid label, g) fluorescence overlay of OregonGreen-PTXL and TexasRed-DHPE. (h) Polarized optical microscopy image of PTXL crystals that have phase separated from unsonicated liposomes (5:92:3 initial mole ratio of DOTAP/DOPC/PTXL) five days after hydration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

segregation during mitosis and subsequently activating apoptotic signaling pathways that lead to cell death [3–8]. PTXL is among the most common drugs used to treat ovarian, breast, lung, pancreatic, and other cancers and is included in the World Health Organization's List of Essential Medicines [9–13]. PTXL is commonly administered in the form of Taxol®, where it is solubilized for delivery in Kollifor-EL (formerly Cremophor EL), which causes hypersensitivity reactions and delivers PTXL non-discriminately throughout the body [14–16]. In 2005, nanoparticle albumin-bound PTXL was approved by the FDA (Abraxane®); this formulation is considered to have fewer adverse reactions than Taxol, although there are mixed reports on whether it improves survival outcomes [17–19].

Considering its biochemical mechanism of action, PTXL should be effective against most cancer cells. Therefore, its lack of efficacy against some cancers is likely caused by the inadequacies of the available drug delivery vehicles. There are various examples in the literature that demonstrate that the drug carrier is a contributing factor in determining which cancers a treatment will be effective against. For example, PEGylated liposomal doxorubicin (DOX) has proven to be more effective than conventional DOX administration to treat AIDS-related Kaposi's sarcoma [20]. PTXL in the form of Abraxane appears to be effective to treat metastatic melanoma, whereas the Taxol formulation is not [21]. Thus, development of novel drug delivery agents for established drugs may open new treatment avenues against an expanded range of cancers.

Liposomal nanoparticles (NPs) are versatile drug delivery agents due to their ability to sequester multiple distinct therapeutic molecules, including long and short nucleic acids (electrostatically condensed with membranes) [22], as well as hydrophilic and hydrophobic drugs in different forms (solubilized, crystallized, surface-conjugated) [23]. Because each therapeutic drug molecule possesses unique physical and chemical characteristics, its liposomal carrier must be tailored to these properties to achieve efficient delivery [24].

Studies suggest that longer retention times of an anticancer drug within circulating liposomes leads to greater accumulation of the drug at tumor sites [25,26]. Much of this work has been done using DOX and other drugs that can be loaded efficiently in the interior aqueous pocket of liposomes (e.g. Doxil and Myocet) [27]. To prolong retention of these drugs in liposomes, the current approach is to increase the rigidity of the membrane and thereby reduce its permeability to hydrophilic drugs, by employing lipids with high melting points (e.g. with saturated tails) and including cholesterol.

Hydrophobic drugs, on the other hand, are solubilized by and reside directly in the nonpolar (hydrocarbon chain) region of the membrane (Fig. 1b and c). These drugs, which include PTXL, are quickly expelled from membranes consisting of saturated lipid tails or those that have a high concentration of cholesterol [28–33]. This observation indicates that the maximum loading and residence time of hydrophobic drugs within liposomes are particularly sensitive to the liposome composition. A key concern is that hydrophobic drugs will leach out of liposomes quickly *in vivo* because they reside at the particle boundary rather than the interior, and will subsequently bind to plasma proteins with hydrophobic pockets which act as 'lipid sinks' [23,26,34].

Various studies indicate that liposome–PTXL formulations exhibit lower toxicity compared to Taxol®, may increase the maximum tolerated drug dose, and may improve biodistribution [30,35–38]. One liposomal formulation of PTXL is approved in China (Lipusu®) [39,40], while others are in clinical trials. Composition information for the Lipusu formulation is not publically available. LEP-ETU is in Phase II trials in the United States; it is an anionic lipid-based carrier composed of the neutral lipid DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine), cholesterol, and cardiolipin (90:5:5 mol ratio) with an additional 3 mol% PTXL [41]. EndoTAG-1 is in Phase III trials in Taiwan and has a cationic liposome structure consisting of the univalent cationic lipid 2,3-dioleyloxypropyltrimethylammonium chloride (DOTAP), DOPC, and PTXL (50:47:3 mol ratio) [28]. Other types of PTXL-containing liposomes (e.g. PEGylated, antibody-targeted) have shown some