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Encapsulation, controlled release, and antitumor efficacy of cisplatin delivered in liposomes composed of sterol-modified phospholipids

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ABSTRACT

We employed a recently introduced class of sterol-modified lipids (SML) to produce m-PEG-DSPE containing liposome compositions with a range of cis-platinum content release rates. SML have a cholesterol succinate attached to the phosphatidylglycerol head group and a fatty acid at the 2 position. These compositions were compared to the well-studied liposome phospholipid compositions: mPEG-DSPE/Hydrogenated Soy PC/cholesterol or mPEG-DSPE/POPC/cholesterol to determine the effect of the cis-platinum release extent on C26 tumor proliferation in the BALB/c colon carcinoma mouse model. The release rates of cis-platinum from liposomes composed of SML are a function of the acyl chain length. SML-liposomes with shorter acyl chain lengths C-8 provided more rapid cisplatin release, lower *in vitro* IC50, and were easier to formulate compared to liposomes using traditional phospholipid compositions. Similar to other liposome cis-platinum formulations, the half-life of m-PEG-DSPE SML liposome cisplatin is substantially longer than the free drug. This resulted in a higher tumor cisplatin concentration at 48 h post-dosing compared to the free drug and higher Pt-DNA adducts in the tumor. Moreover, the maximum tolerated dose of the liposome formulations where up to four fold greater than the free drug. Using X-ray fluorescence spectroscopy on tumor sections, we compared the location of platinum, to the location of a fluorescence lipid incorporated in the liposomes. The liposome platinum co-localized with the fluorescent lipid and both were non-uniformly distributed in the tumor. Non-encapsulated Cis-platinum, albeit at a low concentration, was more uniformly distributed thorough the tumor. Three liposome formulations, including the well-studied hydrogenated HSPC composition, had better antitumor activity in the murine colon 26 carcinoma model as compared to the free drug at the same dose but the SML liposome platinum formulations did not perform better than the HSPC formulation.

1. Introduction

Cisplatin is a potent chemotherapeutic used to treat a variety of cancers including ovarian, lung, and colon; but treatment dosage and thus efficacy has been hampered by severe toxicities (Oberoi et al., 2013; Kieler-Ferguson et al., 2013). Limited success has been achieved in designing new platinum derivatives or carrier mediated delivery. Polymer platinum delivery has focused on the design of chelators that release platinum slowly enough to reduce toxicity, but quickly enough to maintain activity (Kieler-Ferguson et al., 2013; Hang et al., 2016). In contrast, liposomal delivery has focused on formulation conditions, which includes methods of encapsulation as well as lipid formulations that would provide appropriate platinum release *in vivo*. The instability of platinates makes drug delivery more challenging. Cisplatin activity is maintained or increased by limiting light exposure, high NaCl concentrations to prevent the loss of chloride and limit isomerization, or exposure to deactivating sulfur nucleophiles (Kieler-Ferguson et al., 2013; Hang et al., 2016). To this end, a variety of different lipid formulations have been explored for cisplatin (Newman et al., 1999; Woo et al., 2008; Schroeder et al., 2009; Hirai et al., 2010; Stathopoulos, 2010; Zisman et al., 2011), oxaliplatin (Dragovich et al., 2006; Suzuki et al., 2008; Tippayamontri et al., 2011) and other platinates (Mori et al., 1996; Wheate et al., 2010). Currently there are no FDA-approved platinum liposomes and four formulations are in clinical trials: Lipoplatin (cisplatin) and Lipoxal (oxaliplatin), developed by Regulon (Fantini et al., 2011) and two others (Hang et al., 2016).

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SPI-077 (SEQUUS) is a well-known cisplatin formulation removed from clinical trials due to lack of activity. SPI-077 is composed of hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and m-PEG-DSPE at a 51:44:5 M ratio; this formulation exhibited superior results in animal models compared to both cisplatin and other cisplatin liposome formulations (Newman et al., 1999). Despite SPI-077 resulting in appreciable Pt uptake in tumor tissue and improved survival outcomes in mice, there was minimal activity in Phase I/II human clinical trials (Harrington et al., 2001; Rosenthal et al., 2002; Meerum Terwogt et al., 2002; White et al., 2006). Zamboni et al. used microdialysis in mice to track the bioavailability of cisplatin and found that only a small percentage of the drug was freely diffusible, despite the improved tumor accumulation (Zamboni et al., 2004). These results were confirmed in a similar efficacy study by Bandak and coworkers, where <10% of cisplatin was released from liposomes and no improvement in efficacy was observed in lung, lymphoma and melanoma models (Bandak et al., 1999).

Additional clinical trials with liposome based platinum compounds are recently reviewed although here again some of these formulations are well tolerated but have not yet exhibited high antitumor activity compared to the non-encapsulated platinum in either animal models or in human trial (Oberoi et al., 2013; Hang et al., 2016).

These data suggested to us that a new liposome formulation which increases the bioavailability of cisplatin in the tumor, is still needed. Thus, we explored the potential of sterol modified lipids or SMLs (Huang and Szoka, 2008; Huang et al., 2009; Foglia et al., 2011; Kohli et al., 2014a; Kohli et al., 2014b) to deliver cisplatin, as previous studies have shown variable content release based upon chain length and lipid composition. The SMLs were designed to improve bilayer properties by covalently linking cholesterol the PC lipid head group, replacing one of the acyl chains, and eliminating cholesterol transfer between the liposome and biomembranes which may contribute to catastrophic instability of traditional fluid phospholipid/cholesterol compositions such as POPC/cholesterol mixtures. This study illustrates how modifications to lipid composition can influence drug release, animal toxicity, tumor uptake, and anti-tumor efficacy. The intratumoral distribution of the liposome and the platinum reinforces the previous findings that the liposome carrier and the encapsulated platinum are co-localized in the tumor periphery.

2. Experimental methods

2.1. Materials

Materials were used as obtained from commercial sources unless otherwise noted. Cisplatin was purchased from Sigma-Aldrich. Lipids (Hydrogenated Soy Phosphatidylcholine (HSPC)), PalmitoylOleoyl Phosphatidyl choline (POPC), dipalmitoylphosphatidylcholine (DPPC) and methoxypolyethyleneglycol-2000-distearoylphosphatidylethanolamine (m-PEG-DSPE) were purchased from Avanti Polar Lipids, kindly synthesized by Dr. Zhaohua Huang, or synthesized as previously described (Huang and Szoka, 2008; Huang et al., 2009). Cholesterol (Chol) was recrystallized from methanol prior to use. SPEX CertiPrep Ultralene Film (4 μm) was purchased from Fisher Scientific. The platinum ICP standard was purchased from VHG Labs. Chloroform was removed under reduced pressure using a rotary evaporator.

2.2. General liposome preparation

Prior to liposome formation, lipids were dissolved in chloroform, evaporated to form a thin film, and dried overnight at room temperature under a high vacuum. Lipid mixtures are abbreviated as listed in Table 1. Various formulation conditions, as described below and in the supplemental materials, were explored to make liposomes. Following formation, liposomes were sequentially extruded through a 200 nm and 100 nm polycarbonate membrane 11 times each at 60 °C, held at 60 °C for 15 min and then cooled to room temperature. Any non-encapsulated cisplatin that precipitated and was removed by filtration through a 0.4 Spectrum 25 mm PTFE sterile syringe filter, and the liposomes were then dialyzed for 24 h against 100 volumes of HEPES buffer (10 mM HEPES, 150 mM NaCl. pH 7.4). Average liposome diameter and zeta potential were determined by dynamic light scattering measurements (Malvern Instruments Zetasizer Nano ZS) (Table 1).

2.3. Quantification of platinum in liposomes

Cisplatin loading in the liposomes was measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Perkin Elmer Optima 7000 DV Optical Emission Spectrometer). Liposomes (50 μL) were diluted to 6 mL with a 5% HNO_3 solution and allowed to stand at room temperature for 1 h. Samples were measured in triplicate for their Pt emission at 214.423 nm.

2.3.1. Liposome formulations

2.3.1.1. Ethanol injection method (Peleg-Shulman et al., 2001). This method was used to prepare liposomes for all *in vitro* and *in vivo* studies. Cisplatin (8.5 mg/mL) was dissolved in 0.9% NaCl at 60 °C, while the lipids were dissolved in ethanol (100 mg/mL) at 60–70 °C. With a prewarmed syringe, the ethanolic mixture (100 μL) was rapidly injected into the cisplatin solution (900 μL) and allowed to stir covered for 1 h at 60 °C. In certain instances, the volume of the preparation was increased to 5 mL total. Formulation continued as described above. Size: 100–132 nm, Platinum loading: 1–1.7 mg cisplatin/10 mg lipids. Other methods including: DMSO, Chaotropic solvents and remote loading were employed to improve the solubility or encapsulation of Pt in liposomes but the ethanol procedure (Peleg-Shulman et al., 2001) was the most reliable and efficient of the variations we examined (Supplemental Information).

2.3.1.2. Platinum release experiments. Cisplatin liposomes diluted into fetal bovine serum (FBS) final concentration 30 vol% were aliquoted into Eppendorf tubes and placed in a 37 °C shaker. At various times over 48 h, a sample was passed through a Sepharose GL-6B column and the liposome fraction collected. We used this separation method because platinum can interact with serum proteins, it was important to separate both free cisplatin and the cisplatin that had interacted with serum proteins from the liposomes. The liposome fraction was

Table 1
Lipid formulations and physical characterization.

Name	Lipid Composition	Molar Ratio	Diameter (nm)	PDI	Zeta potential (mV)
HSPC	HSPC:Chol:m-PEG-DSPE	51:44:5	125	0.04	−2.2
POPC 51	POPC:Chol:m-PEG-DSPE	51:44:5	132	0.06	−2.2
POPC 80	POPC:Chol:m-PEG-DSPE	80:15:5	110	0.06	−2.3
C8Chems	C8Chems:m-PEG-DSPE	95:5	100	0.08	−2.4
C18.1Chems	C18.1Chems:m-PEG-DSPE	95.5	115	0.04	−1.1
DChems	DiChems:m-PEG-DSPE	95:5	130	0.09	−2.4
DChems/DPPC	DiChems:DPPC:m-PEG-DSPE	32:63:5	119	0.1	−2.6