Thermosensitive Depot-Forming Injectable Phosphatidylcholine Blends Tailored for Localized Drug Delivery

JUSTIN GRANT, PAYAM ZAHEDI, ANTONIA TSALLAS, CHRISTINE ALLEN

Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

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ABSTRACT: A thermosensitive depot-forming system was developed for sustained and localized delivery of the anticancer drug, paclitaxel. The formulation is injectable as a melt slightly above the body temperature and forms a solid depot upon cooling to 37°C. The thermosensitive system was prepared by blending various combinations of phosphatidylcholines at specific weight ratios solubilized in laurinaldehyde. Of the blends investigated, distearoyl–phosphatidylcholine (DSPC) and egg–phosphatidylcholine (ePC) were found to be most miscible. A liquid-to-gel phase transition temperature (T_C) of 39°C was observed for the 70:30 (w/w) DSPC–ePC blend and a T_C of 38.4°C with the addition of paclitaxel. Blends containing higher concentrations of ePC had a greater degree of swelling and weight loss. Furthermore, microscopy revealed an increase in porosity and erosion as the amount of ePC was increased in blends incubated in biologically relevant media. DSPC–ePC blends provided sustained release of paclitaxel over a 30-day period and the rate of drug release increased as the amount of ePC increased. Overall, the relationships established between the composition and properties of the blend may be employed to tailor the thermosensitive injectable formulation for localized chemotherapy of solid tumors. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3623–3631, 2013

Keywords: controlled release; drug delivery systems; formulation; injectables; cancer chemotherapy; phospholipids

INTRODUCTION

The standard therapy for most solid cancers consists of cytoreductive surgery followed by systemic chemotherapy. However, systemic delivery of anticancer agents has proven to be inadequate in some cases due to nonspecific distribution of drug resulting in low concentrations in solid tumors and toxicity to healthy tissues.[1] The microenvironment within tumors also obstructs the uptake and distribution of anticancer agents. Tumor properties that hinder drug delivery include disorganized tumor vasculature, high interstitial fluid pressure, and development of multidrug resistance.[2] Methods such as drug modifications and the use of new carrier systems have been explored to circumvent these issues.[3] For example, nanosized drug carriers have been developed to exploit the enhanced permeability and retention effect,[4] resulting in significant improvements in tumor-drug concentrations.[5,6] Implants and microparticles have also been employed to achieve localized and sustained chemotherapy.[7–9] Localized drug delivery can result in higher drug concentrations at tumor sites, provide extended drug exposure, and minimize systemic toxicities. In particular, injectable systems including polymer-based gels and pastes have been examined for localized cancer therapy due to their ease of preparation and administration as well as improved patient compliance and comfort.[10,11]

Various types of *in situ* forming injectable depot systems exist and can be differentiated by their mechanism of depot formation.[12] These systems include thermoplastic pastes, which are injected as a melt into the body and then form a semisolid depot upon cooling to physiological temperature (i.e., 37°C). Conventionally, these systems have been composed of polyesters such as poly(lactide) and poly(glycolide).[10] However, polyester-based materials have been shown to produce a foreign body response and form acidic by-products that can accelerate the degradation of drugs.[13–15] Recently, polysaccharides[16,17] and block copolymers including pluronics[18,19] have been employed as thermosensitive materials. Most of the injectable thermoplastic pastes that have been evaluated to date have a high melting temperature.[20–22] These systems need to be injected at high temperatures, which can lead to the formation of scar tissue and necrosis at the injection site.[10] For these reasons, there is a need to develop injectable thermoplastic pastes that are biocompatible and have melting temperatures only slightly above the body temperature.

In this study, thermosensitive injectable formulations composed of phosphatidylcholines were developed for localized and sustained delivery of the hydrophobic anticancer agent paclitaxel. Natural phospholipids, such as phosphatidylcholines, have been used to prepare drug formulations due to their established biocompatibility.[23] Previously, we demonstrated that local delivery of paclitaxel *via* an implantable film composed of phosphatidylcholine and the natural polymer chitosan resulted in enhanced efficacy compared to conventional bolus injections of chemotherapy in ovarian cancer mouse models.[24–28] The need for invasive surgical implantation led us to pursue an injectable formulation with similar performance-related properties. Blends of phosphatidylcholines of varying chain length (i.e., C12–C18) and degrees of saturation were used to prepare the formulations. Specifically, blends composed of distearoyl–phosphatidylcholine (DSPC) mixed with either egg–phosphatidylcholine (ePC), dimyristoyl–phosphatidylcholine (DMPC), dipalmitoyl–phosphatidylcholine (DPPC), or dilauroyl–phosphatidylcholine (DLPC) were solubilized in laurinaldehyde (LA). Differential scanning calorimetry (DSC) was used to assess the thermosensitivity and miscibility of the blends. The molecular

Correspondence to: Christine Allen (Telephone: +416-946-8594; Fax: +416-978-8511; E-mail: cj.allen@utoronto.ca)

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interactions between the lipids were also examined by X-ray diffraction (XRD) analysis. The swelling, weight loss, and erosion characteristics of the blends were measured and observed by scanning electron microscopy (SEM). Finally, the release profile of paclitaxel from the blends was measured in biologically relevant media at 37°C. Overall, slight changes in the composition of the blend led to significant differences in the physicochemical and performance-related properties, enabling customization of the thermal and physical stabilities, and drug-release profiles of the formulations for local therapy.

MATERIAL AND METHODS

Materials

DMPC, DPPC, DLPC and ePC were purchased from Northern Lipids (Burnaby, British Columbia, Canada). DSPC was obtained from Genzyme (Cambridge, Massachusetts). LA and albumin were purchased from Sigma–Aldrich (Oakville, Ontario, Canada). Anhydrous ethanol was obtained from Commercial Alcohols Inc. (Brampton, Ontario, Canada). Paclitaxel was purchased from Polymed Therapeutics Inc. (Houston, Texas).

Preparation of the Blends

DSPC was blended with DLPC, DMPC, DPPC, or ePC in various weight ratios [100:0, 80:20, 70:30, 60:40 (w/w)] for a total of 100 mg of phospholipid and solubilized in 200 μL of LA [1:1.7 (w/w) lipid-to-LA ratio]. Each mixture was vortexed for 5 min and then heated to 60°C until the phosphatidylcholines were fully dissolved. For drug-loaded blends, 4 mg of paclitaxel was added to the blend and then dissolved in 200 μL LA, followed by vortexing and heating as above, to achieve a drug-to-lipid ratio of 1:25 (w/w). A clear gastight 2.5 mL syringe (Hamilton Company, Reno, Nevada) was used for all injections. The formulations were injected prior to solidification, as visualized in the syringe.

Thermal Analysis

Q100 differential scanning calorimeter (TA Instruments, New Castle, Delaware) was used for thermal analysis. All samples were prepared to include the specified phospholipids in addition to 200 μL of LA. Samples (5–10 mg) were weighed in aluminum pans and sealed. Each sample was heated to 90°C and then cooled to −30°C at a rate of 5°C per min under nitrogen purge. The cycle was repeated to remove thermal history and used to analyze the liquid-to-gel phase transition temperature. TA universal analysis software was used for the analysis of the thermograms.

Swelling and Weight Loss

All samples were drug free and prepared as above with 100 mg of the DSPC–ePC blend in 200 μL of LA. The samples were heated to 60°C and prior to solidification, the formulation was injected into 5 mL of phosphate buffered saline (PBS) (pH 7.4, 0.01 M) solution containing 45 mg/mL albumin incubated at 37°C. The initial weight of each blend was recorded (W_i) and at specific time points the blends were removed from the solution, rinsed with distilled water, and blotted dry to obtain the swelling weight (W_s). For weight-loss determination, the blends were lyophilized (Freezone 4.5, Labconco, Kansas City, Missouri) for 24 h and weighed (W_d). The degree of swelling (1) and weight loss (2) were calculated from the following equations:

$$\text{Degree of swelling}(\%) = (W_s - W_d)/W_d \times 100 \quad (1)$$

$$\text{Weight loss}(\%) = (W_i - W_d)/W_i \times 100 \quad (2)$$

Turbidity

The samples were prepared as above with 100 mg of each phospholipid blend (drug free) in LA, heated to 60°C, and then injected into 5 mL of PBS (pH 7.4, 0.01 M) solution containing 45 mg/mL albumin incubated at 37°C. At specific time points, an aliquot of 2.5 mL was removed and analyzed by UV–vis spectroscopy (Varian Inc., Palo Alto, California) at a wavelength of 600 nm. Following each measurement, the 2.5 mL aliquot was returned to the sample solution.

X-ray Diffraction Analysis

X-ray diffraction patterns of DSPC–ePC blends (drug free) in LA were obtained using a Siemens D5000 0/20 diffractometer (Siemens, Mississauga, Ontario, Canada) with Cu Kα source operating at 50 kV. The secondary beam was monochromatized by a Kevex solid detector. Patterns were obtained using a step width of 1° 2θ between 3° and 35° 2θ at ambient temperature. A parallel Goebel-mirror beam from a Cu Kα source was employed and the data was collected on transmission mode with a two-dimensional detector.

Scanning Electron Microscopy

The surface morphology of the drug-free lipid blends prepared in LA, following 1 day and 49 days of incubation at 37°C in 5 mL PBS (pH 7.4, 0.01 M) solution containing 45 mg/mL albumin, was observed using a Hitachi S-570 (Hitachi, Tokyo, Japan) scanning electron microscope operating under vacuum at a voltage of 15 kV. Blends were rinsed with distilled water and lyophilized prior to mounting onto aluminum slotted heads for imaging.

In Vitro Drug Release

In vitro drug release was measured for DSPC in LA and DSPC–ePC blends (i.e., 80:20, 70:30, and 60:40 w/w) in LA loaded with 2% (w/v) paclitaxel and injected into vials containing 5 mL 0.01M PBS (pH 7.4), 45 mg/mL albumin, and incubated at 37°C. At set time points, 2.5 mL aliquots were removed for analysis by high-performance liquid chromatography (HPLC) and replaced with 2.5 mL fresh release media. An Agilent series 1100 HPLC (Agilent Technologies, Mississauga, Ontario, Canada) equipped with a Waters XTerra MS C18 5 μm (4.6 × 250 mm) column, XTerra® MS C18 5 μm (3.9 × 20 mm) guard column, and Waters dual absorbance detector 2487 (Waters, Mississauga, Ontario, Canada) with ChemStation software was used for analysis. The wavelength of detection used was 227 nm. A mobile phase of 52% H_2O and 48% acetonitrile was used. An injection volume of 100 μL per sample with a flow rate of 0.6 mL/min was applied. Docetaxel (10 μg/mL) was used as an internal standard for all HPLC analysis. Extraction of paclitaxel from *in vitro* release samples was achieved by first adding 300 μL of each sample to a vial containing docetaxel as the internal standard. The vial was vortexed for 5 min and then 5 mL *tert*-butyl methyl ester was added followed by 10 min of additional mixing. The