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Research paper

Layer-by-layer assembly of hierarchical nanoarchitectures to enhance the systemic performance of nanoparticle albumin-bound paclitaxel

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ABSTRACT

Although protein-bound paclitaxel (PTX, Abraxane®) has been established as a standard PTX-based therapy against multiple cancers, its clinical success is limited by unfavorable pharmacokinetics, suboptimal biodistribution, and acute toxicities. In the present study, we aimed to apply the principles of a layer-by-layer (LbL) technique to improve the poor colloidal stability and pharmacokinetic pattern of nanoparticle albumin-bound paclitaxel (*nab*-PTX). LbL-based *nab*-PTX was successfully fabricated by the alternate deposition of polyarginine (pARG) and poly(ethylene glycol)-block-poly (L-aspartic acid) (PEG-*b*-PLD) onto an albumin conjugate. The presence of protective entanglement by polyamino acids prevented the dissociation of *nab*-PTX and improved its colloidal stability even at a 100-fold dilution. The combined effect of high nanoparticle internalization and controlled release of PTX from LbL-*nab*-PTX increased its cytotoxicity in MCF-7 and MDA-MB-231 breast cancer cells. LbL-*nab*-PTX consistently induced apoptosis in approximately 52% and 22% of MCF-7 and MDA-MB-231 cancer cells, respectively. LbL assembly of polypeptides effectively prevented exposure of PTX to the systemic environment and thereby inhibited drug-induced hemolysis. Most importantly, LbL assembly of polypeptides to nab-PTX effectively increased the blood circulation potential of PTX and improved therapeutic efficacy via a significantly higher area under the curve $(AUC)_{0-\infty}$. We report for the first time the application of LbL functional architectures for improving the systemic performance of *nab*-PTX with a view toward its clinical translation for cancer therapy.

1. Introduction

Breast cancer is the leading cause of cancer-related death in women worldwide (WHO, 2016a, 2016b). Despite significant advances in treatment, breast cancer has a poor 5-year survival rate of <15%, with a third of the patients eventually developing metastases (GLOBOCAN, 2008; WHO, 2016a, 2016b). The primary treatment option for breast cancer is surgery/radiotherapy followed by chemotherapy. As opposed to treatment by surgery and radiotherapy, the use of chemotherapeutic compounds inhibits cell differentiation and potentially induces systematic apoptosis of cancer cells (Gennari et al., 2005; Ma et al., 2010). Because of the importance of adjuvant chemotherapy, it is important to develop novel anticancer drug formulations for effective breast cancer treatment.

Paclitaxel (PTX) is a potent anticancer drug indicated for the treatment of advanced and metastatic breast cancers (Gluck, 2015). It inhibits the depolymerization of microtubules and arrests cells in the mitotic phase (Kumar et al., 2015; Shimizu et al., 2014). The clinical benefits of PTX therapy are challenged by high hydrophobicity, poor water solubility (0.3–0.5 μg/mL), and non-selectivity (Lee et al., 2008; Singla et al., 2002; Yoncheva et al., 2012). Therefore, the commercially available form of PTX (Taxol®) is formulated in a mixed solution of polyoxyethylene castor oil (Cremophor EL®) and dehydrated ethanol (Zheng et al., 2014). However, the use of Cremophor EL® results in severe acute toxicity including hypersensitivity, anaphylaxis, neurotoxicity, and nephrotoxicity. Further, the Taxol® formulation does not improve the drug's erratic pharmacokinetics and non-selective Biodistribution (Campos et al., 2014). Clinically, Taxol® is administered as an intravenous infusion at 135–175 mg/m^2 (4–24 h), with a reported

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terminal half-life ($t_{1/2}$) of ~5 h and C_{max} of ~60 μg/Ml (Ko et al., 2014). The immediate clearance of PTX from systemic circulation leads to subtherapeutic effects with acute toxicity concerns (Liu et al., 2015). Moreover, much of the PTX has been observed in vital organs.

In order to overcome the limitations of Taxol®, the United States Food and Drug Administration (USFDA) has approved a Cremophor EL®-free formulation of 130 nm PTX-bound albumin (Abraxane®) for effective breast cancer treatment (Elsadek and Kratz, 2012). The rationale for using Abraxane® is to protect PTX's pharmacological activity while eliminating the adverse effects of Cremophor EL® (Elzoghby et al., 2012). Studies of Abraxane® have shown relatively higher therapeutic efficacy and reduced systemic toxicity than those of Taxol®. Although the use of Abraxane® has resulted in an initial positive response, it is still hindered by many limitations, including hypersensitivity reactions and hematological side effects (Gradishar, 2006; Sparreboom et al., 2005). Most importantly, the poor colloidal stability of Abraxane® results in a poor pharmacokinetic pattern and PTX's rapid elimination from the blood circulation (Ruttala and Ko, 2015a, 2015b). Active PTX dissociates from albumin within 3–4 h of intravenous administration and possesses a C_{max} value of 13 μg/mL after 3 h, which drops to 1 μg/mL after 4 h with a single dose of 260 mg/m^2 in human patients (Ruttala and Ko, 2015a, 2015b). Therefore, efforts to improve PTX's colloidal stability in the systemic circulation and sustain its blood levels represent a major objective of breast cancer treatment.

Layer-by-layer (LbL) nanoparticles (NPs) fabricated by the deposition of polycations and polyanions on the colloidal surface in an alternative manner are becoming increasingly important in cancer-targeting applications (De Cock et al., 2010). LbL NPs offer some unique benefits, including controlled drug release, improved stability of the nanocarrier in circulation, increased storage stability, and robustness (Hammond, 2012; Becker et al., 2010). In this study, we aimed to apply the principles of the LbL technique to improve the poor colloidal stability and pharmacokinetic pattern of nanoparticle albumin-bound PTX (*nab*-PTX). There is evidence to suggest that long blood circulation times and long NP half-lives allow drugs to accumulate in malignant tumor tissues via enhanced permeation and retention (EPR) effects (Ramasamy et al., 2014a, 2014b, 2014c, 2014d), which would improve the clinical benefits of LbL-based *nab*-PTX if applicable. The alternative assembly of complementary materials protects the labile *nab*-PTX from the outer physiological environment and preserves the original structure for the efficient delivery of anticancer drugs. The application of the LbL architectural approach to *nab*-PTX presents daunting challenges, including maintaining colloidal stability of resulting LbL-functionalized *nab*-PTX (LbL-nPTX) in physiological conditions, maintaining the final LbL-NP size, and enhancing the ability of polyethylene glycol (PEG) to confer long blood circulation times. Considering these challenges, we selected poly(arginine) (pARG) and poly(ethylene glycol)-block-poly (L-aspartic acid) (PEG-*b*-PLD) as the cationic and anionic materials, respectively. pARG is a positively charged synthetic polyamino acid known to enhance the cellular uptake of NPs, and PEG-*b*-PLD provides the much needed antifouling, colloidal stability, and pH-responsiveness (Ramasamy et al., 2014a, 2014b, 2014c, 2014d; Szyk-Warszyńska et al., 2014). Owing to the definite primary structure and high content of amino acids, albumin allows sequential deposition of oppositely charged polyamino acids (pARG and PEG-*b*-PLD). Few studies have described the application of an LbL approach to drug delivery systems, and the main emphasis has been the barrier-controlled release of the core content. For the first time, we have applied LbL functional architectures to improve the systemic performance of *nab*-PTX with a view to its clinical translation for cancer therapy.

To this end, we sought to improve the *in vivo* stability and pharmacokinetic patterns of *nab*-PTX by applying the principles of the LbL technique to an albumin conjugate. Specifically, *nab*-PTX was assembled with 6 alternating layers of pARG and PEG-*b*-PLD. The present study describes the mechanistic basis of LbL-NPs and gives a detailed characterization of physicochemical parameters including size, shape, colloidal stability, and release kinetics. Furthermore, cytotoxicity analysis and hemolysis potential of individual formulations are reported. Importantly, the systemic stability of *nab*-PTX and LbL-*n*PTX was studied in an animal model, and respective pharmacokinetic parameters were modeled by non-compartmental analysis using WinNonlin software.

2. Material and methods

2.1. Materials

PTX was obtained from Shaanxi Top Pharm Chemical Co. Ltd. (Xi'an, China). Albumin and pARG (MW: 5000) were purchased from Sigma-Aldrich, China. PEG_{113}-*b*-PLD_{10} (MW: 6700) was procured from Alamanda Polymers (Huntsville, AL, USA). The block lengths were 113 and 10 repeating units for PEG and PLD, respectively. All other chemicals were of reagent grade and used without further purifications.

2.2. Fabrication of lbL-albumin conjugates

Albumin nanoparticles were prepared by a desolvation method. In brief, 1 mg of albumin was dissolved in 1 mL of water and stirred for 15 min. The albumin solution was filtered through a 0.45 μm filter and the pH was adjusted to 9. Separately, 10 mg of PTX was dissolved in 1 mL of 100% ethanol. PTX solution (10 μL) was added to 200 μL of albumin solution and stirred for 10 min. The solution was sonicated for 2 h. The ethanol concentration was less than 1% in the final formulations.

LbL assembly was performed on the albumin conjugates as previously reported. Briefly, the albumin conjugate, pARG, and PEG-*b*-PLD solutions were prepared at a final concentration of 1 mg/mL. Aliquots of each polyamino acid solution (2–4 μL) were alternately added to the albumin conjugate, vortexed for 10 min, and then sonicated for 8 min. No intermittent washing or centrifugation steps were performed, ensuring no loss of polymer or NPs. The exact amount of polymer needed to cover the NP surface was calculated by careful titration. The encapsulation efficiency and drug loading of the NP formulations were determined using Agilent 1200 series high-performance liquid chromatography (HPLC) after ultrafiltration using an Amicon® centrifugal filter device (molecular weight cut off of 10,000 Da; EMD Millipore, Billerica, MA, USA). A mobile phase comprised of acetonitrile and 0.1% formic acid in water (35:65 (VOR) and 60:40 (PTX), v/v) with a flow rate of 1.0 mL/min was used. The eluent was measured using an UV–vis detector at 227 nm.

2.3. Hydrodynamic size, zeta potential, and polydispersity

Particle size, zeta potential, and polydispersity index (PDI) were measured by the dynamic light scattering (DLS) technique. NP dispersions were suitably diluted with distilled water and measured using a Zeta Sizer (Nano ZS, Malvern Instruments, U. K.). Each experiment was performed in triplicate.

2.4. Morphological analysis

The morphology of *nab*-PTX and LbL-*n*PTX was determined by transmission electron microscopy (TEM, H7600, Hitachi, Tokyo, Japan) at an accelerating voltage of 100 kV. A drop of NP dispersion