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Polymeric micelles for the pH-dependent controlled, continuous low dose release of paclitaxel

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ABSTRACT

Poly(ethylene glycol)-*block*-poly(aspartate-hydrazide) (PEG-p(Asp-Hyd)) was modified using either levulinic acid (LEV) or 4-acetyl benzoic acid (4AB) attached via hydrazone bonds. Paclitaxel (PTX) conjugated to the linkers formed PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX). PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX) assemble into unimodal polymeric micelles with diameters of 42 nm and 137 nm, respectively. PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX) at a 1:1 and 1:5 molar ratio assemble into unimodal mixed polymeric micelles with diameters of 85 and 113 nm, respectively. PEG-p(Asp-Hyd-LEV-PTX) micelles release LEV-PTX faster at pH 5.0 than at pH 7.4 over 24 h. At pH 7.4 mixed polymeric micelles at 1:5 ratio show no difference in LEV-PTX release from PEG-p(Asp-Hyd-LEV-PTX) micelles. Mixed polymeric micelles at 1:5 molar ratio gradually release LEV-PTX at pH 5.0, with no release of 4AB-PTX. PEG-p(Asp-Hyd-LEV-PTX) micelles and mixed polymeric micelles exert comparable cytotoxicity against SK-OV-3 and MCF-7 cancer cell lines. In summary, mixed polymeric micelles based on PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX) offer prospects for pH-dependent release of PTX, offering a novel prodrug strategy for adjusting its pharmacokinetic and pharmacodynamic properties for cancer therapy. If successful this delivery system offers an alternative new mode of delivery for paclitaxel with a new scope for its efficacy along with a minimal synthetic framework needed to accomplish this.

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1. Introduction

Paclitaxel is one of the most broadly active compounds available for the treatment of human malignancy, with activity demonstrated in cancers of the ovary, breast, lung, head and neck, esophagus, bladder, testis, endometrium, and possibly hematological and pediatric malignancies [1]. PTX is also a strong inhibitor of the endothelial-cell function relevant to angiogenesis at low concentrations [2,3]. New research has shown that PTX has anti-angiogenic activity by inhibiting vascular endothelial-cell proliferation, motility and cord/tube formation at extremely low concentrations (e.g., picomolar) [3,4]. The anti-angiogenic efficacy of PTX seems to be amplified by administering comparatively low doses of PTX [5]. Due to the low aqueous solubility of PTX, 0.3 μM [6], it is commercially available as Taxol® in a vehicle composed of 1:1 of Cremophor EL® (CrEL) (polyoxyethylated castor oil) and ethanol. Side effects caused by CrEL include hypersensitivity reactions, nephrotoxicity, and neurotoxicity [7–9]. Further, CrEL at clinically relevant concentrations nullifies the anti-angiogenic activity of paclitaxel [3]. Taxol® is also limited by its short term physical stability upon dilution as PTX tends to precipitate out of the aqueous media [10]. Other problems associated with ethanol and CrEL include leaching of plasticizer from polyvinyl chloride infusion bags and sets [11]. Thus, there are many strategies for improved vehicles for the delivery of PTX with high anti-tumor and anti-angiogenic activity with reduced formulation related adverse effects, culminating in the recent approval of Abraxane®, albumin nanoparticles that permit higher doses of PTX over Taxol®, owing to an absence of CrEL [12].

Amphiphilic block copolymers (ABCs) assemble into polymeric micelles that are nanoscopic, have a core/shell architecture, and have wide potential in cancer therapy for the tumor targeting of PTX by the enhanced permeability and retention (EPR) effect [13]. Poly(ethylene glycol)-*block*-poly(β-benzyl L-aspartate) (PEG-PBLA) is an ABC that has been used as a starting point for polymeric micelles for the delivery of chemotherapy [14,15]. A major advantage of PEG-PBLA is its ease in side chain chemistry, allowing side chain variation for drug solubilization and/or prodrug design. The

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replacement of benzyl ester protecting groups on PEG-PBLA with 4-phenyl-1-butanol leads to polymeric micelles that increase the water solubility of PTX to ca. 10 mg/mL, increase its plasma half-life from ca. 1 to 6 h in mice, and increase PTX levels in solid tumors by the EPR effect [16]. NK105 is in Phase II clinical trials for stomach cancer therapy [16]. Recent research on doxorubicin (DOX) outlines an alternative prodrug strategy for PTX: replacement of benzyl groups on PEG-PBLA with hydrazine permits the coupling of DOX via hydrazone linkages (PEG-p(Asp-Hyd-DOX)). PEG-p(Asp-Hyd-DOX) micelles are stable at pH 7.4 and release DOX under acidic conditions, associated with the extracellular environment in hypoxic solid tumors and endosomal/lysosomal pathway of cells [17–20]. However, PTX lacks an aldehyde or ketone group for a hydrazone linkage, mandating a linker for pH-sensitive release.

The goal of this proposed research is to prepare polymeric micelles that can solubilize PTX and release this anti-cancer agent in a pH-dependent manner. In addition, these polymeric micelles will provide a formulation platform that can be tailored for specific regimens for PTX administration to maximize its anti-angiogenic activity, which is based on administering comparatively low doses of PTX. Taking a hydrazone prodrug strategy, we propose coupling an aliphatic linker, levulinic acid (LEV) or an aromatic linker, 4-acetyl benzoic acid (4AB), on poly(ethylene glycol)-*block*-poly(aspartate-hydrazide) (PEG-p(Asp-Hyd)) to introduce carboxylic acid groups for attachment of PTX by an ester bond. Following the synthesis we expect these polymers to self-assemble into micelles that will release PTX in a pH-dependent manner. PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX)will also be used to form mixed micelles and determine PTX pH-dependent release profiles from these micelles. We have hypothesized that mixed polymeric micelles composed of PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX) would have a composite release profile for PTX, reflecting the rate of hydrazone hydrolysis of LEV-PTX and 4AB-PTX in mixed polymeric micelles (Fig. 1). If successful, we anticipate that these polymeric micellar delivery system offer new prospects for moderating the pH-dependent release of PTX and for enhancing its pharmacokinetic and pharmacodynamic properties, aiming for advances in cancer therapy for solid tumors.

2. Materials and methods

2.1. Materials

α-Methoxy-ω-amino-poly(ethylene glycol) (PEG-NH_2) (M_n 12,000 g/mol, PDI = 1.03) was purchased from NOF Corporation (Tokyo, Japan). PTX was obtained from LKT laboratories Inc (St. Paul, MN). SK-OV-3 (Human Caucasian ovary adenocarcinoma) and MCF-7 cell lines (Human breast adenocarcinoma) were purchased from American Type Culture Collection (Manassas, VA). All other materials and reagents were obtained from Sigma–Aldrich Inc (Milwaukee, WI) or Fisher Scientific Inc (Fairlawn, NJ).

2.2. Methods

2.2.1. Synthesis of amphiphilic diblock copolymer PEG-PBLA

PEG-PBLA was synthesized as previously reported [21,22]. Briefly, β-benzyl-L-aspartate *N*-carboxyanhydride (BLA-NCA) was prepared using Fuchs-Farthing method, triphosgene 5.76 g (19.4 mmol) was added to 10 g (44.8 mmol) of β-benzyl-L-aspartate in dry tetrahydrofuran (THF) (150 mL), and the reaction was allowed to proceed at 40 °C until the solution became clear. BLA-NCA was purified by recrystallization from hexane and purity was confirmed by measuring its melting point at 129.5 °C. Ring opening polymerization of BLA-NCA (1.7 g, 6.82 mmol) was initiated from the terminal primary amine group of PEG-NH_2 (2.0 g, 0.16 mmol). The polymerization was carried out under argon atmosphere in anhydrous DMSO (7.0 mL) at 40 °C for 2 days. PEG-PBLA was obtained after precipitation from diethyl ether followed by freeze drying from benzene.

2.2.2. Synthesis of PEG-p(Asp-Hyd)

PEG-p(Asp-Hyd) was synthesized as previously reported [17,18,20]. Briefly, PEG-PBLA (1.0 g, 0.05 mmol) was dissolved in anhydrous N,N-dimethylformamide (DMF) (10 mL), and anhydrous hydrazine (1.2 eq with respect to benzyl groups) was added to the polymer solution under argon atmosphere. The reaction was allowed to proceed at 40 °C for 1 h followed by polymer precipitation in diethyl ether. PEG-p(Asp-Hyd) was dialyzed against 0.25% ammonia solution then 0.025% ammonia solution and freeze-dried to obtain the product, PEG-poly(Asp-Hyd). The structure of PEG-poly(Asp-Hyd) was confirmed using ^{1}H NMR (500 MHz, DMSO-d_6).

2.2.3. Synthesis of PEG-p(Asp-Hyd-LEV) and PEG-p(Asp-Hyd-4AB)

The pH labile hydrazone bond was formed via the reaction of the hydrazine groups on the PEG-p(Asp-Hyd) with the ketone group on LEV or 4AB, resulting in the formation of PEG-p(Asp-Hyd-LEV) or PEG-p(Asp-Hyd-4AB), respectively. PEG-p(Asp-Hyd) (750 mg, 0.046 mmol) was dissolved in 15 mL anhydrous dimethyl sulfoxide (DMSO). 4AB or LEV (1.2 eq with respect to hydrazide group) was dissolved in anhydrous DMSO and added to the polymer solution under argon. The reaction was allowed to proceed for 48 h at 45 °C. PEG-p(Asp-Hyd-LEV) or PEG-p(Asp-Hyd-4AB) was collected from diethyl ether, solubilized in DMF, and dialyzed against DMF using regenerated cellulose (MWCO: 3000 g/mol). Polymers were collected from diethyl ether and then freeze-dried from benzene.

2.2.4. Synthesis of PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX)

PEG-p(Asp-Hyd-LEV) (200 mg, LEV = 0.376 mmol) or PEG-p(Asp-Hyd-4AB) (200 mg, 4AB = 0.352 mmol) was conjugated to PTX (600 mg, 0.468 mmol) using N,N′-diisopropylcarbodiimide (DIC; 56 mg, 0.44 mmol) as coupling agent in the presence of 4-dimethylaminopyridine (DMAP; 21.7 mg, 0.17 mmol). The reaction was carried out in (dichloromethane (DCM): DMF) (5:1) (7.0 mL) at 25 °C for 48 h. PEG-p(Asp-Hyd-LEV-PTX) or PEG-p(Asp-Hyd-4AB-PTX) was collected by evaporating DCM, and the residue was diluted in 5.0 mL DMF. The polymer solution was dialyzed against 1.0 L DMF (DMF was changed 3 times) using regenerated cellulose (MWCO: 6–8000 g/mol). The polymer was precipitated in diethyl ether, freeze-dried

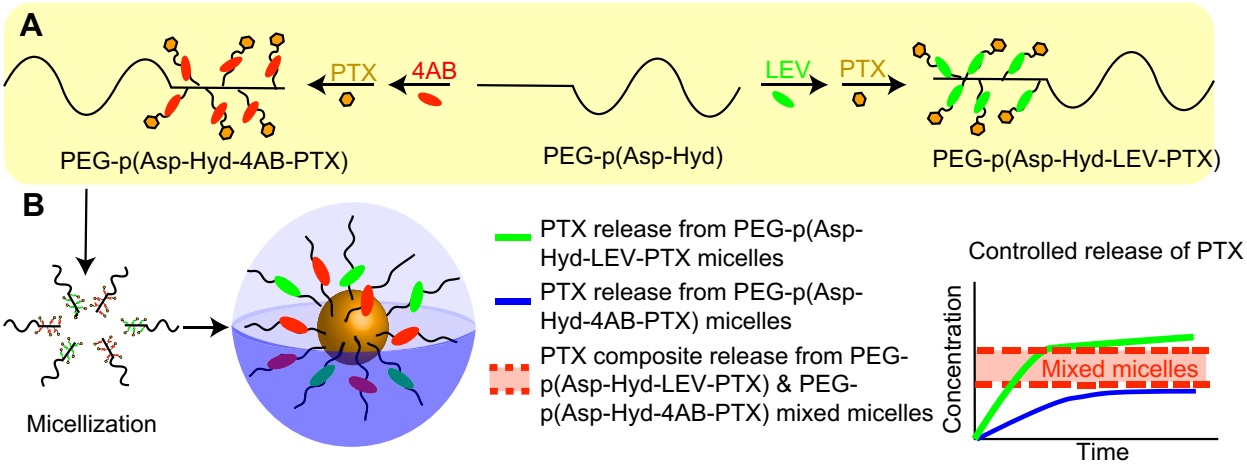

Fig. 1. Synthesis scheme for PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX) (A); A sketch of a mixed polymeric micelle and expected PTX release profiles from PEG-p(Asp-Hyd-LEV-PTX), PEG-p(Asp-Hyd-4AB-PTX) micelles and mixed polymeric micelles (B).