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Multi-drug loaded polymeric micelles for simultaneous delivery of poorly soluble anticancer drugs

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ABSTRACT

Current clinical and preclinical anticancer formulations are limited by their use of toxic excipients and stability issues upon combining different drug formulations. We have found that poly(ethylene glycol)-*block*–poly(D,L lactic acid) (PEG-*b*–PLA) micelles can deliver multiple poorly water-soluble drugs at clinically relevant doses. Paclitaxel (PTX), etoposide (ETO), docetaxel (DCTX) and 17-allylamino-17-demethyoxygeldanamycin (17-AAG) were solubilized individually in PEG-*b*–PLA micelles. Combinations of PTX/17-AAG, ETO/17-AAG, DCTX/17-AAG and PTX/ETO/17-AAG were also solubilized in PEG-*b*–PLA micelles. PEG-*b*–PLA micelles were characterized in terms of drug loading, size, stability and drug release. All anticancer agents in all combinations were all solubilized at the level of mg/mL and were stable for 24 h in the 2- and 3-drug combination PEG-*b*–PLA micelles. The stability of the 2- and 3-drug combination PEG-*b*–PLA micelles was due to the presence of 17-AAG. *In vitro*, $t_{1/2}$ values for 2- and 3-drug combination PEG-*b*–PLA micelles spanned 1–5 h. PEG-*b*–PLA micelles offer a promising alternative for combination drug therapy without formulation related side effects.

1. Introduction

Polymeric micelles are nanoscopic core/shell structures usually formed through the self-assembly of amphiphilic block copolymers (ABCs) [1]. ABC micelles have a hydrophobic core surrounded by a hydrophilic outer shell. The inner core can be used as a storage site for poorly water-soluble drugs and can act as a nano-depot for these agents. This drug-loaded inner core is protected by a biocompatible hydrophilic outer shell. Furthermore, heterogeneous functionalities can be introduced in each domain to facilitate drug loading and targeting. Over the past few years, ABC micelles have been used as drug carriers for poorly water-soluble drugs that result in improved pharmacokinetics (PK) for these drugs. These properties along with a proven safety record in humans has lead to a research spurring it as an alternate to commercial anticancer formulations [1–4]. Examples of poorly water-soluble drugs in recent studies involving ABC micelles include amphotericin B, cyclosporin A, 4-hydroxyphenylretinamide, irinotecan, paclitaxel (PTX), propofol, and rapamycin [5–11].

Poorly water-soluble drugs like anticancer agents in preclinical development, e.g. 17-allylamino-17-demethoxygeldanamycin (17-AAG), and many in clinical practice, e.g. PTX, docetaxel (DCTX), and etoposide (ETO), require safe vehicles for drug solubilization and IV infusion. However, vehicles for IV drug infusion are often toxic, e.g. Cremophor EL (CrEL), and hamper progress in combination drug therapy involving poorly water-soluble anticancer agents. Combination therapy with current excipients runs the risk of precipitation and additive or synergistic toxicity caused by two or more vehicles for drug solubilization, e.g. CrEL for PTX and DMSO/lipid for 17-AAG. Hypersensitivity reactions occur in approximately 40% of patients that receive CrEL and PTX despite pre-medication with corticosteroids and histamine antagonists. CrEL-induced hypersensitivity reactions cause discontinuation of drug therapy, and are life-threatening in 1–3% of patients even with pre-medication with corticosteroids and histamine antagonists [12]. Other serious toxicities associated with CrEL use include nephrotoxicity and neurotoxicity [13–15]. In preclinical research, intraperitoneal injection of Taxol® and 17-AAG in rapid succession results in swift deaths in mice, mandating a 20 min separation of injections for combination drug therapy [16]. It is noted that the most common grade 2 toxicity associated with a 1- or 1.5-h IV infusion of 17-AAG/DMSO/lipid was bad odor and nausea in a phase II clinical trial for melanoma [17]. In a phase I clinical trial involving 17-AAG and trastuzumab, grade 3 hypersensitivity reactions in two patients associated with a 2-h IV infusion of 17-AAG/CrEL/propylene glycol/ethanol resulted in discontinuation in the clinical trial despite pre-medication, noting a partial response in one of the patients [18].

Common IV formulations for anticancer agents include the use of CrEL and ethanol for PTX, ethanol and polyoxyethylene sorbitan monooleate (Tween 80) for DCTX [19] and Tween 80 for ETO [20]. As noted, 17-AAG is a poorly water soluble and required DMSO/lipid or CrEL for administration in phase I/II clinical trials [21]. Thus, there is a clear need to find alternate formulations and vehicles to deliver these agents without contributing to the side effects experienced by patients on chemotherapy.

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PEG-*b*–PLA, an ABC, assembles readily in water into micelles and has been shown to raise the solubility of PTX from approximately 1 μg/mL to 10 mg/mL [9]. PEG-*b*–PLA is less toxic than CrEL. However, a recent phase II clinical trial in metastatic breast cancer patients showed that PTX dosed as part of PEG-*b*–PLA micelles, without pre-medication with corticosteroids and histamine antagonists, does induce hypersensitivity reactions, albeit less severely than CrEL. PEG-*b*–PLA micelles increase the maximum tolerated dose of PTX in humans in comparison to CrEL, enhancing its anti-tumor efficacy [22]. There is also evidence that PEG-*b*–PLA micelles impart linear PK for PTX, strongly contrasting with CrEL that induces a non-linear PK profile for PTX, i.e. lowering its clearance with dose escalation [12]. Using these PEG-*b*–PLA micelles to raise the solubility of various anticancer agents is a potential delivery option, facilitating ease of entry into clinical trials in the cancer arena.

However, due to the heterogeneity of cancer cells as well as acquired drug resistance, single agent therapy is limited and combination chemotherapy has become a standard regimen to treat cancer patients. To be specific, drug combinations are beneficial in the view of retarding occurrence of resistant cell lines and wide coverage against multiple cell lines, resulting in maximum cell killing effect within acceptable toxicity [23]. Synergistic drug combinations produce an even greater response rate or survival time than is possible with each drug used alone at its optimum dose. For example, 17-AAG, a prototype Hsp90 inhibitor had synergistic effects with a broad range of anticancer agents in different tumor cell lines [21,24]. 17-AAG causes a remarkable combinatorial depletion of multiple oncogenic proteins, e.g. Akt, ErbB-2, and Hif-1α, causing a blockage of cancer-causing and survival pathways, and there is keen interest in the combination of chemotherapy and 17-AAG [25].

In the case of 17-AAG and PTX it has been shown that 17-AAG sensitizes cancer cells to apoptosis induced by PTX, a mitotic inhibitor with anti-neoplastic activity, when the drugs are given together or when 17-AAG treatment was followed by exposure to PTX [26,27]. The combination of these two drugs was synergistic in different cancer cell lines and in mice [16,25,26,28]. Additionally in mice bearing H358 human non-small-cell lung cancer xenografts PTX cytotoxicity was enhanced by 5–22 fold when combined with 17-AAG [29]. The combination of PTX and 17-AAG was also evaluated in humans and showed enhanced efficacy and better tolerability profile as compared to PTX alone [30]. In another case 17-AAG has also enhanced the activity of ETO, a topoisomerase II inhibitor, *in vitro* and *in vivo* [31]. The combination of 17-AAG and ETO showed synergism in leukemia cells [32]. Another study demonstrated the combination of 17-AAG and ETO decreased the IC_{50} of ETO by 10 fold in four different pediatric acute lymphoblastic leukemia cell lines [33].

However, despite the advantages of combination chemotherapy, one of the main problems associated with clinical use is the complicated regimens that must be administered to patients. As most anticancer drugs are poorly water soluble and utilize toxic excipients to enhance their solubility, combining two or three drugs can be challenging in clinical practice, owing to compatibility and stability issues [23]. Thus, using PEG-*b*–PLA micelles to rationally design and deliver chemotherapeutic regimens instead of single anticancer agents might be a better approach to overcome these formulation related and clinical challenges. In our previous work, we successfully solubilized 17-AAG in PEG-*b*–PLA micelles [34]. The PK profile of 17-AAG in these micelles was similar to CrEL in rats, without the attendent toxicity observed with the CrEL formulation (no deaths versus 35% mortality for CrEL) [34]. Based on this platform our goal is to create PEG-*b*–PLA micellar systems that can simultaneouly deliver multiple anticancer agents, like PTX, DCTX, or ETO by co-solublizing them with 17-AAG to generate safer, more stable formulations for potentially synergistic combination chemotherapy (Fig. 1).

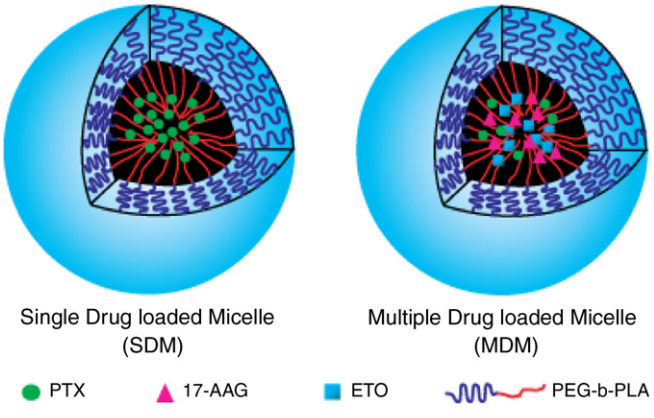

Fig. 1. Schematic representation of single and multiple drug-loaded PEG-*b*–PLA micelles.

2. Materials and methods

2.1. Materials

PEG-*b*–PLA (M_n PEG and PLA are 4.2 K and 1.9 K respectively, PDI = 1.05) was purchased from Advanced Polymer Materials Inc. (Montreal, CAN). Paclitaxel was obtained from LKT laboratories Inc. (St. Paul, MN). Docetaxel and 17-AAG were purchased from LC Laboratories (Woburn, MA). Etoposide, DMSO-d_6 and D_2O were purchased from Sigma-Aldrich Inc. (St. Louis, MO). All other materials were obtained from Fisher Scientific Inc. (Fairlawn, NJ). All reagents were HPLC grade.

2.2. Methods

2.2.1. Preparation and characterization of drug-loaded PEG-b–PLA micelles

Single drug micelles (SDMs) were prepared by adding 2.0 mg of PTX, DCTX, ETO or 17-AAG and 15 mg of PEG-*b*–PLA in a 5.0 mL round bottom flask. The drug–polymer mixture was dissolved in 0.50 mL acetonitrile (ACN). The ACN was removed at 60 °C under reduced pressure on a rotary evaporator resulting in the formation of a homogenous film. The drug–polymer film was rehydrated with 0.50 mL of DD H_2O at 60 °C with gentle agitation resulting in a clear solution of drug-loaded PEG-*b*–PLA micelles. The micellar solution was filtered using a 0.45 μm filter, and the micelles were characterized in terms of size and loading by Dynamic Light Scattering (DLS) and HPLC respectively.

PEG-*b*–PLA was also used to prepare multiple drug micelles (MDMs) with different drug combinations of PTX/17-AAG, DCTX/17-AAG, ETO/17-AAG and PTX/ETO/17-AAG. MDMs were prepared similarly to the SDMs by mixing 2.0 mg of each drug with 15 mg of the polymer. The procedure for the preparation and characterization of these micelles was identical to the SDMs.

Drug(s) to polymer w/w percent was calculated for SDM and MDM. PEG-*b*–PLA micelles were freeze dried, weighed and the amount of drug(s) in the freeze dried sample was quantified by HPLC. The drug w/w percent was calculated as the mass of the drug(s) to the mass of polymer in the freeze dried sample multiplied by 100.

2.2.2. Quantification of drug loading in SDM and MDM by reverse phase HPLC

The content of drug loaded in PEG-*b*–PLA micelles was quantified by reverse phase HPLC. The HPLC system used for quantifying was a Shimadzu prominence HPLC system (Shimadzu, JP), consisting of a LC-20AT pump, SIL-20AC HT autosampler, CTO-20AC column oven and a SPD-M20A diode array detector. A sample of 10 μL was injected into a Zorbax SB-C8 Rapid Resolution cartridge (4.6×75 mm, 3.5 micron, Agilent). The column temperature was maintained at 40 °C throughout