Acta Biomaterialia 58 (2017) 399–412

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Folate-targeted polymersomes loaded with both paclitaxel and doxorubicin for the combination chemotherapy of hepatocellular carcinoma

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ARTICLE INFO

Article history:
Received 11 January 2017
Received in revised form 8 June 2017
Accepted 12 June 2017
Available online 13 June 2017

Keywords:
Polymersomes
Co-delivery
Paclitaxel
Doxorubicin
Folate-receptor targeted
Combination chemotherapy

ABSTRACT

Combination chemotherapy is a promising method of improving cancer treatment, but the distinct pharmacokinetics of combined drugs and non-specific drug distribution slow down the development in the clinic. In this study, folate (FA) receptor-targeted polymersomes with apparent bilayered lamellar structure were successfully developed to co-encapsulate a hydrophobic-hydrophilic chemotherapeutic drug pair (PTX and DOX) in a single vesicle for enhancing the combination chemotherapeutic effect. Hydrophobic PTX was loaded into the thick hydrophobic lamellar membrane by the self-assembly of triblock copolymer PCL_{8000}-PEG_{8000}-PCL_{8000}, while hydrophilic DOX was encapsulated into the hydrophilic reservoir using a trans-membrane ammonium sulfate gradient method. *In vitro* release study indicated that the drugs were released from the polymersomes in a controlled and sustained manner. Cellular uptake study indicated that FA-targeted Co-PS had higher internalization efficiency in FA receptor-overexpressing BEL-7404 cells than non-targeted Co-PS. *In vitro* cytotoxicity assay demonstrated that FA-targeted Co-PS exhibited less cytotoxic effect than free drug cocktail, but suppressed the growth of tumor cells more efficiently than non-targeted Co-PS. *Ex vivo* imaging biodistribution studies revealed that FA-targeted Co-PS led to highly efficient targeting and accumulation in the BEL-7404 xenograft tumor. Furthermore, the *in vivo* antitumor study showed that the combination chemotherapy of polymersomes to BEL-7404 tumor via intravenous injection was superior to free drug cocktail treatment, and the FA-targeted Co-PS exhibited significantly higher tumor growth inhibition than non-targeted Co-PS group. Therefore, the newly developed FA-targeted co-delivery polymersomes hold great promise for simultaneous delivery of multiple chemotherapeutics and would have great potential in tumor-targeting and combination chemotherapy.

Statement of Significance

Combination chemotherapy is a promising method of improving cancer treatment, but the distinct pharmacokinetics of combined drugs and non-specific drug distribution slow down the development in the clinic. In our study, novel folate-targeted co-delivery polymersomes (Co-PS) were successfully developed to encapsulate a hydrophobic-hydrophilic chemotherapeutic drug pair (paclitaxel and doxorubicin) into the different compartments of the vesicle. *In vivo* studies revealed that the combination chemotherapy of polymersomes to BEL-7404 xenograft tumor via intravenous injection was superior to free drug cocktail treatment, and the FA-targeted Co-PS exhibited significantly higher tumor growth inhibition than non-targeted Co-PS group. Therefore, the newly developed FA-targeted co-delivery polymersomes hold great promise for simultaneous delivery of multiple chemotherapeutics and would have great potential in tumor-targeting and combination chemotherapy.

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http://dx.doi.org/10.1016/j.actbio.2017.06.017
1742-7061/

1. Introduction

Cancer is a major cause of death worldwide and chemotherapy is still one of the frontline strategies employed in the treatment of many types of tumors [1]. Among the chemotherapeutic drugs, doxorubicin (DOX) and paclitaxel (PTX) are two of the most effective first-line drugs with excellent activity against various solid tumors. PTX is an antimitotic agent that inhibits microtubules disassembly and stabilizes microtubules by preventing depolymerization during cell division, while DOX is an anthracycline antibiotic that works by intercalating DNA and inhibiting further nucleic acid biosynthesis [2,3]. Due to their different action mechanism on cancer cells, the combination of DOX and PTX has shown increased tumor regression rate and enhanced patient survival rate in comparison to single agent therapy [4–6]. However, severe dose-limiting toxic effects of neuropathy, neutropenia, and cardiotoxicity occurred due to poor targeting efficiency of free drugs, which has become a serious problem in clinical cancer treatments [4]. Meanwhile, clinical studies demonstrated a non-coordinated distribution of PTX and DOX when administered in cocktail solution, resulting from the distinct pharmacokinetics of combined drugs [7,8]. Thus, it is imperative to develop a more effective combination strategy to coordinate the pharmacokinetics of different drug molecules, improve drug concentration and biodistribution within the tumor, enhance combinatorial anti-tumor efficacy while reducing adverse effects.

Over the past few decades, the nano-scaled drug carriers based on amphiphilic copolymers have provided a promising combination strategy by simultaneous loading multiple drugs with different mechanisms of action to the same tumor cells via a single vehicle. Several nano-scaled carriers including polymeric micelles, polymersomes, prodrug nanoparticles, nanogels, have been designed to achieve controlled and synchronized release of multiple therapeutic agents for cancer combination therapy [9–13]. Among these drug carriers, polymersomes have attracted rapidly growing interest in recent years as an ideal platform to simultaneously encapsulate multiple drugs as their structure is analogous to liposomes, both containing hydrophilic and hydrophobic chambers to encapsulate water soluble and insoluble anticancer agents [14–17]. In comparison with liposomes where the lipid properties are limitedly adjustable, the membrane thickness, mechanic strength and flexibility, and the permeability and fluidity of the polymersomes can be easily modulated by changing the composition and molecular weight of the block copolymers [18–21]. As versatile carriers, polymersomes hold superior advantages including greater colloidal stability, improved storage capabilities, tunable physicochemical behaviour, prolonged circulation time, improved controlled release properties and ease of functionalization [22,23].

Amphiphilic block copolymers in water can self-assemble into various types of mesoscopic structures, such as micelles (spherical, prolate, or oblate) and polymersomes [24]. The types of structure formed are dependent on the mass or volume ratio of hydrophilic block to total copolymer ($\int$), molecular weight of the copolymer, as well as the employed preparation methods [25]. Our previous study indicated that amphiphilic triblock copolymer PCL-PEG-PCL with different $\int$PEG (w) can form polymeric micelles and polymersomes, separately, using thin film re-hydration and sonication method. When the molecular weight of both PEG block and PCL block was 8000 g/mol, PCL-PEG-PCL ($\int$PEG = 33%) was found to form polymersomes with clear hollow vesicular structure [26]. The bilayer membrane of the polymersomes revealed a hydrophobic core thickness of around 30 nm, which was significantly greater than liposomes bi-layers (3-nm hydrophobic cores), providing higher loading capacity and extended retention of hydrophobic molecules within the polymersomes' membrane. In addition, unlike polymeric micelles which can only integrate hydrophobic compounds within their hydrophobic core unless covalent linking or strong electrostatic interaction strategies are employed for loading hydrophilic compounds, polymersomes can simultaneously encapsulate hydrophilic molecules in their aqueous interior core [27].

To our knowledge, there are only a handful of reports in the literature on polymersome-mediated co-delivery of DOX and PTX as cancer therapy. Chen and coworkers developed polymersomes from poly (ethylene glycol) – poly (2,4,6-trimethoxybenzylidene pentaerythritol carbonate)(PEG-PTMBPEC) diblock copolymer and studied simultaneous encapsulation and release of PTX and DOX from the polymersomes [28]. Colley and colleagues formulated polymersomes based on poly 2-(methacryloyloxy) ethyl phosphorylcholine (PMPC) – poly 2-(diisopropylamino) ethyl methacrylate (PDPA) to encapsulate PTX and DOX, and studied the *in vitro* anticancer therapy of the co-delivery system [29]. These studies focused on *in vitro* characterization of novel polymersomes for co-delivery, but the *in vivo* feasibility of these approaches was not evaluated. Discher and colleagues are the first one to develop polymersomes for PTX and DOX co-delivery and study the *in vivo* anti-tumor effect [13,30]. Their polymersomes consisted of PEG – (polylactic acid, PLA) and PEG-polybutadiene blend. Their study indicated that a single systemic injection of the dual drug combination in polymersome at maximum tolerated dose led to shrinkage of tumors in mice. Inspired by the work of Discher and colleagues, we have formulated a chemically distinct, targeted polymersome system based on PCL-PEG-PCL triblock copolymer and lipid-PEG conjugates with folate as tumor targeting ligand, aiming to achieve both active tumor targeting and synergistic chemotherapy of DOX and PTX *in vitro* and *in vivo* (Scheme 1). The novel biodegradable polymersomes were designed with following superior properties: (1) multiple drugs with different solubility and action mechanism can be administered simultaneously; (2) the pharmacokinetic profiles of the different drug can be altered to be the same using the polymersomes; (3) the drug ratio and concentration at the desirable tumor site can be altered via changing the ratio of drugs; (4) high accumulation in tumors can be achieved via prolonged blood circulation and EPR effect; (5) superior anti-tumor efficacy can be realized by the combined active tumor-targeting and synergistic chemotherapy.

2. Materials and methods

2.1. Materials

Poly (ethylene glycol) (PEG, Mn = 8000), ε-Caprolactone and coumarin-6 were purchased from Sigma-Aldrich. DSPE-PEG (2000) Folate was obtained from Avanti Polar Lipids. Doxorubicin (DOX, >98.0% purity) was purchased from Dalian Biological Technology Co., Ltd. Paclitaxel was purchased from Sichuan Jiufeng Natural Pharmaceutical Co. Ltd. Taxol® (30 mg/5 mL) was obtained from Beijing Union Pharmaceutical Factory. CellTiter 96® Aqueous One Solution Cell Proliferation Assay (MTS) was purchased from Promega Company. 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) solution was purchased from Beyotime Institute of Biotech. Fetal bovine serum and related cell culture medium were from Invitrogen.

2.2. Preparation of the co-delivery polymersomes

Co-delivery polymersomes (Co-PS) conjugated with or without folate (FA) ligand were formulated by a thin film re-hydration method plus remote loading procedures. Briefly, for FA-targeted PTX-loaded polymersomes, DSPE-PEG (2000) Folate, PTX, and PCL_{8000}-PEG_{8000}-PCL_{8000} copolymer were dissolved in dichloromethane (DCM) in a round-bottom flask. A PTX-copolymer-lipid