Acta Biomaterialia 9 (2013) 9330–9342

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Doxorubicin-loaded amphiphilic polypeptide-based nanoparticles as an efficient drug delivery system for cancer therapy

Shixian Lv [a,b,1], Mingqiang Li [a,b,1], Zhaohui Tang [a], Wantong Song [a,b], Hai Sun [a], Huaiyu Liu [c,*], Xuesi Chen [a,*]

[a] Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[b] University of Chinese Academy of Sciences, Beijing 100039, China
[c] Laboratory Animal Center, Jilin University, Changchun 130012, China

ARTICLE INFO

Article history:
Received 10 April 2013
Received in revised form 25 July 2013
Accepted 9 August 2013
Available online 17 August 2013

Keywords:
Poly(amino acids)
Doxorubicin hydrochloride
Electrostatic interaction
pH sensitive
Drug delivery

ABSTRACT

An amphiphilic anionic copolymer, methoxy poly(ethylene glycol)-*b*-poly(L-glutamic acid-*co*-L-phenylalanine) (mPEG-*b*-P(Glu-*co*-Phe)), with three functionalized domains, was synthesized and used as a nanovehicle for cationic anticancer drug doxorubicin hydrochloride (DOX·HCl) delivery via electrostatic interactions for cancer treatment. The three domains displayed distinct functions: PEG block chain for prolonged circulation; poly(phenylalanine) domain for stabilizing the nanoparticle construct through hydrophobic/aromatic interactions; and the poly(glutamic acid) domain for providing electrostatic interactions with the cationic drug to be loaded. The copolymer could self-assemble into micellar-type nanoparticles, and DOX was successfully loaded into the interior of nanoparticles by simple mixing of DOX·HCl and the copolymer in the aqueous phase. DOX-loaded mPEG-*b*-P(Glu-*co*-Phe) nanoparticles (DOX-NP) had a superior drug-loading content (DLC) (21.7%), a high loading efficiency (almost 98%) and a pH-triggered release of DOX. The size of DOX-NP was ~140 nm, as determined by dynamic light scattering measurements and transmission electron microscopy. In vitro assays showed that DOX-NP exhibited higher cell proliferation inhibition and higher cell uptake in A549 cell lines compared with free DOX·HCl. Maximum tolerated dose (MTD) studies showed that DOX-NP demonstrated an excellent safety profile with a significantly higher MTD (15 mg DOX kg^{-1}) than that of free DOX·HCl (5 mg DOX kg^{-1}). The in vivo studies on the subcutaneous non-small cell lung cancer (A549) xenograft nude mice model confirmed that DOX-NP showed significant antitumor activity and reduced side effects, and then enhanced tumor accumulation as a result of the prolonged circulation in blood and the enhanced permeation and retention effect, compared with free DOX, indicating its great potential for cancer therapy.

Introduction

Cancer is one of the most common causes of death in the world. Despite tremendous advances in cancer diagnosis and treatment, the cure for cancer is still a Gordian knot [1]. Chemotherapy remains the primary treatment for cancer among conventional modalities. However, many chemotherapeutic agents, such as cytotoxic drugs, can freely diffuse in both normal and neoplastic cells. This induces non-specific drug distribution in the body and severe systemic side effects, and therefore chemotherapy often results in an unsatisfactory curative effect due to the side effects of the drugs [2,3]. Nanosized drug carriers using natural or artificial polymers appear to be a promising and reliable approach to cancer treatment, with enhanced antitumor efficacy and reduced toxic side effects [4]. Compared with conventional systemic chemotherapies, nanosized anticancer carriers have favorable properties based on well-preprogrammed structures, such as high drug-loading capacity, high stability by avoiding rapid clearance by the renal and reticuloendothelial systems (RES) and minimized drug loss during blood circulation [5], enhanced accumulation in tumors through the enhanced permeability and retention (EPR) effect [6] and facilitated drug release triggered by environmental stimuli in the tumor sites (e.g., temperature [7–9], pH [10–12] and glutathione [13,14]). Several nanomedicines have been approved for clinical use, such as Doxil and Abraxane, which have been used as effective treatments for metastatic breast cancer and recurrent ovarian cancer [15,16].

Among all the nanosized drug carriers, self-assembled polymeric nanoparticles of poly(ethylene glycol)-*b*-poly(amino acids) (PEG-PAA) have emerged as one of the most promising platforms for improved antitumor drug delivery and have been widely studied in preclinical and clinical trials, owing to their excellent biodegradability and biocompatibility [17]. These self-assembled nanoparticles consist of a hydrophilic PEG shell and a PAA core

* Corresponding authors. Tel./fax: +86 431 85262112 (X. Chen).
E-mail addresses: liuhuaiyu@jlu.edu.cn (H. Liu), xschen@ciac.ac.cn (X. Chen).
[1] These authors contributed equally to this paper.

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.08.015

incorporating antitumor drugs. The PEG shell can prevent nanoparticles from adsorption of protein and recognition by the phagocyte system, and in that way, prolong blood circulation time [5,18]. PAA is highly biocompatible, biodegradable and non-toxic, and can easily be synthesized by a well-established ring-opening polymerization (ROP) method. In addition, PAA has versatile functional groups such as carboxyl, amino, hydroxyl and thiol groups, which offer great benefits in modifying the chemical structure of the core for efficient drug incorporation and controllable drug release properties [19–22]. Accordingly, several polymeric nanoparticles of PEG-PAA incorporating doxorubicin, paclitaxel, SN-38 and cisplatin exhibited significant antitumor efficacy with appreciably lowered toxicity compared with free drugs, and are currently under clinical evaluation [23–26].

However, most of these nanomedicines are achieved by chemical conjugation and/or physical entrapment of hydrophobic drugs into the nanoparticles. The nanosized drug delivery systems based on electrostatic PEG-PAA block copolymers and the charged hydrophilic antitumor drugs are still rarely investigated. Previously, the present authors prepared a ionomer complex formed by anionic methoxy poly(ethylene glycol)-*b*-poly(L-glutamic acid) (mPEG-*b*-PLG) and cationic DOX·HCl in the therapy of non-small-cell lung cancer (NSCLC) [27]. The results demonstrated that mPEG-*b*-PLG was an efficient carrier for delivering DOX into solid tumors and achieved improved pharmacokinetics, biodistribution and then reduced toxicity compared with free DOX·HCl. However, both PEG and PLG segments of the block copolymer are hydrophilic in a physiological environment, and there are no other groups to stabilize the complex formulation; therefore, the DOX-loaded mPEG-*b*-PLG system revealed unsatisfactory cellular uptake and lower cell proliferation inhibition activity compared with free DOX·HCl. Recent studies confirmed that the stability of the nanoparticles was a key factor for a successful drug delivery system [28,29]. By increasing the overall hydrophobicity of the block copolymer in the nanoparticles, the uptake of the drug carriers by the tumor cells can be greatly enhanced, which will lead to a significant increase in anticancer activity.

Herein, a drug delivery system is developed based on PEG-PAA loaded with hydrophilic DOX·HCl. As an anionic polymer to facilitate self-assembled nanoparticle formation via electrostatic interactions with DOX, a novel A(BC) copolymer composed of three monomeric units, mPEG-*b*-P(Glu-*co*-Phe), is used. Amphiphilic anionic mPEG-*b*-P(Glu-*co*-Phe) block copolymers are expected to undergo spontaneous self-assembly in aqueous solutions, and three monomeric units of the copolymer are expected to perform specified functions for efficient drug delivery. PEG is used for the prolonged circulation of nanoparticles for effective EPR effects. Anionic poly (glutamic acid) serves as the functional group of the copolymer to provide the electrostatic interaction with cationic DOX·HCl. The incorporation of phenylalanine units into the copolymer is considered to enhance hydrophobic/aromatic interactions within the nanoparticle core.

Compared with other types of nanoparticles and microparticles for drug delivery, the present nanoparticles have the following advantages. (1) Most of the reported drug-loaded nanocarriers are obtained through hydrophobic drug encapsulation procedures, which involve the dissolution of the polymeric carrier and drug in an organic solvent and the subsequent removal of the organic solvent by either dialysis or solvent evaporation. For example, DOX·HCl is usually neutralized by excess triethylamine and makes doxorubicin hydrophobic in organic solvents (*N*,*N*-dimethylformamide (DMF), tetrahyrofuran, chloroform or dimethylsulfoxide (DMSO)). Nevertheless, the trace residual triethylamine and solvent may do harm to the human body. Conversely, the present mPEG-*b*-P(Glu-*co*-Phe)/DOX polyion complex nanoparticles can be prepared by simple mixing of the drug and the copolymers in aqueous solution, without the use of harmful organic solvents. This approach will make the drug encapsulation procedure much simpler and safer. Additionally, electrostatic interactions between the polymers and the drugs would offer great benefits for drug release. Since the environmental acidity has a great impact on the surface charge of the electrostatic block copolymers and also affects electronic interactions between the polymers and the drugs, such electrostatic polymer/drug complexes could be designed for intracellular pH-sensitive drug delivery systems [30–32]. (2) Compared with other electrostatic polymer/drug polyion complex nanoparticles, the present nanoparticles also have advantages. Unlike most of the reported anionic polymer/DOX·HCl complexes, which were based on non-biodegradable polymers (e.g., poly(methacrylic acid) and poly(acrylic acid)) and devoid of in vivo studies [33,34], the design of the present system is based on biocompatible and biodegradable PEG-PAA. Compared with other biodegradable polymers (e.g., γ-polyglutamic acid and poly(ethylene glycol)-*b*-poly(L-glutamic acid) [27,32], hydrophobic groups were introduced into the copolymers to enhance the construct stability of the nanoparticles towards dissociation by a simple copolymerization procedure of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) and Phe-NCA monomers. Additionally, incorporation of phenylalanine units into the copolymer was also expected to enhance the cell uptake of DOX-NP, which could enhance the overall therapeutic efficacy.

In the present work, the molecular structures, physiochemical properties, self-assembly, stability and loading capacity of mPEG-*b*-P(Glu-*co*-Phe) block copolymer were assessed. The in vitro drug release kinetics, cellular uptake and in vitro cytotoxicity of DOX-NP were further studied. Finally, the antitumor efficacy of DOX-NP in a NSCLC (A549) xenograft model was evaluated. The DOX-loaded electrostatic complex nanoparticles showed reduced systemic toxicity and enhanced antitumor efficacy compared with free DOX·HCl, indicating its great potential for efficient cancer chemotherapy.

2. Experimental section

2.1. Materials

Poly(ethylene glycol) monomethyl ether (mPEG, M_n = 5000) was purchased from Aldrich and used without further purification. BLG-NCA and amino-terminated poly(ethylene glycol) methyl ether (mPEG-NH_2, M_w = 5000 Da) were synthesized as in previous work [35]. L-Phenylalanine-*N*-carboxyanhydride (Phe-NCA, 98%; Shanghai Yeexin Biochem&tech Co., Ltd.) was recrystallized from n-hexane/tetrahydrofuran (1:1) before use. DMF was stored over calcium hydride (CaH_2) and purified by vacuum distillation with CaH_2. Doxorubicin hydrochloride (DOX·HCl) was purchased from Beijing Huafeng United Technology Corporation. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from Sigma and used as received. All the other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received.

2.2. Characterization

^{1}H NMR spectra were recorded on a Bruker AV 400 NMR spectrometer in trifluoroacetic acid-d (CF_3COOD). Number- and weight-average molecular weights (M_w, M_n), and molecular weight distributions (PDI = M_w/M_n) were determined by gel permeation chromatography (GPC) using a Waters GPC system (Waters Styragel HT6E column, with OPTILAB DSP interferometric refractometer as the detector). The eluent was DMF containing 0.01 M lithium

ID	Title	Pages
636	Doxorubicin-loaded amphiphilic polypeptide-based nanoparticles as an efficient drug delivery system for cancer therapy	13