International Journal of Pharmaceutics 511 (2016) 1002–1011

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Biodegradable brush-type copolymer modified with targeting peptide as a nanoscopic platform for targeting drug delivery to treat castration-resistant prostate cancer

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ARTICLE INFO

Article history:
Received 8 May 2016
Received in revised form 29 July 2016
Accepted 8 August 2016
Available online 10 August 2016

Chemical compounds studied in this article:
oligo(ethylene glycol) monomethyl ether methacrylate
ε-caprolactone
benzyl alcohol
bufalin, 3-((2-(methacryloyloxy)ethyl)thio) propanoic acid
N,N′-dicyclohexyl- carbodiimide
4-dimethylaminopyridine
N-hydroxysuccinimide
benzyl 2-methyl-2-(((propylthio) carbonothioyl)thio) propanoate
triethylamine

Keywords:
Biodegradable polymers
Castration-resistant prostate cancer (CRPC)
Bufalin

ABSTRACT

Well-defined amphiphilic tumor-targeting brush-type copolymers, poly(oligo(ethylene glycol) monomethyl ether methacrylate-*co*-G3-C12)-*g*-poly(ε-caprolactone) (P(OEGMA-*co*-G3-C12)-*g*- PCL), were synthesized by the combination of ring-opening polymerization (ROP), reversible addition-fragmentation transfer (RAFT) polymerization and polymer post-functionalization, in which G3-C12 was castration-resistant prostate cancer (CRPC) targeting peptide. The obtained polymers were then employed for the targeted treatment of CRPC by delivering a hydrophobic anticancer drug (bufalin, BUF). Polymerizable monomer, 3-((2-(methacryloyloxy)ethyl)thio)propanoic acid (BSMA) and PCL-based macromolecular monomer (PCLMA) were synthesized at first. RAFT polymerization of OEGMA, BSMA, and PCLMA afforded amphiphilic brush-type copolymers, P(OEGMA-*co*-BSMA)-*g*-PCL. Post-functionalization of the obtained polymers with G3-C12 led to the formation of the final amphiphilic targeting brush-type copolymers, P(OEGMA-*co*-G3-C12)-*g*- PCL. In aqueous media, P(OEGMA-*co*-G3-C12)-*g*-PCL self-assembles into micelles with a hydrodynamic diameter (D_h) of ~66.1 ± 0.44 nm. It was demonstrated that the obtained micellar nanoparticles exhibited good biocompatibility and biodegradability. Besides, BUF-loaded micellar nanoparticles assembled from P(OEGMA-*co*-G3-C12)-*g*-PCL, BUF-NP-(G3-C12), showed a controlled drug release in vitro and improved anticancer efficacy both in vitro and in vivo.

1. Introduction

Prostate cancer (PC) has become the leading cancer for men in the US in recent years (Siegel et al., 2015). Owing to the highly metastasis propensity to distant parts such as bone, lung, and liver, effective treatment of PC remains a formidable challenge. Radical operation, definitive radiotherapy, and hormone manipulation have been used for the treatment of localized prostate cancer and metastatic prostate cancer (Antonarakis et al., 2010; Barve et al., 2014; Maroto et al., 2016; Van Allen and Pomerantz, 2012). However, some of the patients still progressed into hormone refractory prostate cancer (CRPC) and limited clinical strategies are available for effective therapy (Antonarakis et al., 2010; Barve et al., 2014; Van Allen and Pomerantz, 2012). Currently, docetaxel (DTX) is the only FDA-approved drug to combat CRPC. Unfortunately, fast drug-resistant development, severe side effects, and only a small proportion of patients sensitive to this drug restraint its wide clinical application (Niraula and Tannock, 2011; Ryan and Tindall, 2011; Seruga et al., 2011; Seruga and Tannock, 2011). In this context, various new strategies such as nanomedicine, short hairpin RNA, new drugs, targeted therapy, and combination therapy were developed (Engel et al., 2012; Fang et al., 2015; Hoang et al., 2014; Jin et al., 2014a; Martin et al., 2014; Yan et al., 2015; Zhan et al., 2013; Ziada et al., 2004).

Hoang et al. (2014) developed DTX-carboxymethyl cellulose nanoparticles and enhanced anti-tumor activity in murine models of CRPC were observed. It was reported that the nanomedicine exhibited 2–3 fold improvement in survival and enhancements in quality-of-life of the animals over free DTX, implying good

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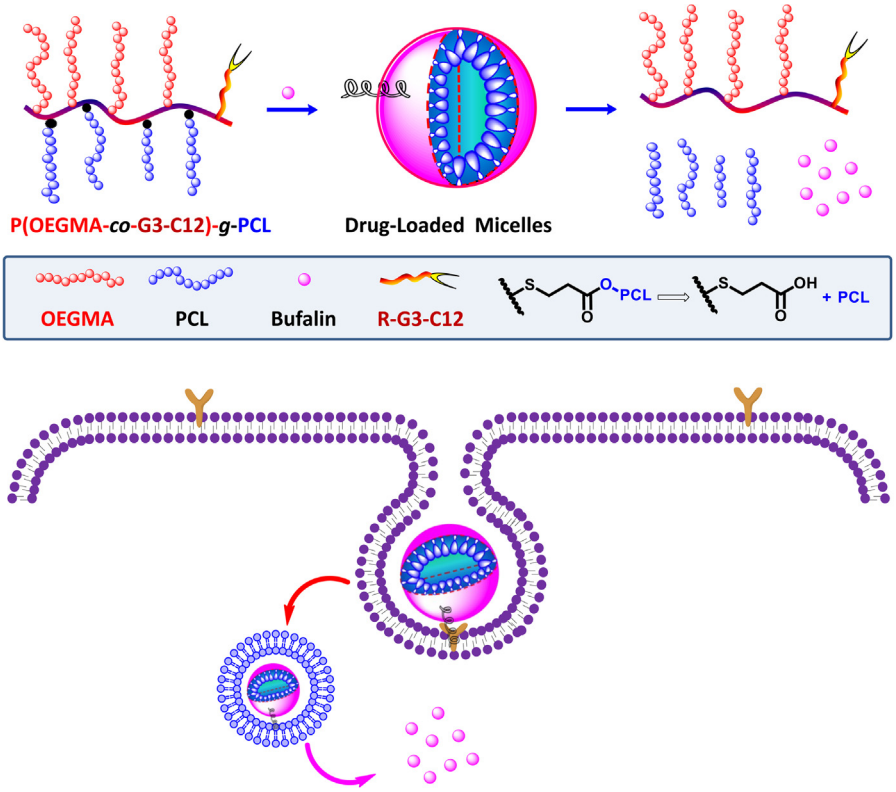
http://dx.doi.org/10.1016/j.ijpharm.2016.08.017
0378-5173/

potential applications. Fang et al. (2015) loaded a novel combimolecule (JDF-12) into the core of the micellar nanoparticles assembled from amphiphilic block copolymers, poly(*D,L*-lactic-*co*-glycolic acid)-*block*-poly(ethylene glycol). The surface of the nanoparticles was decorated with a single-chain antibody that specifically recognizes the extracellular domain of prostate stem cell antigen (PSCA) of CRPC. Yan et al. (2015) employed lipid-polymer hybrid nanoparticles (LPNs) to simultaneously encapsulate DTX and curcumin (CUR) into nanoparticle, showing greater cytotoxicity than single drug-encapsulated LPNs and free drugs.

Moreover, galectin-3 (Gal-3), a carbohydrate binding protein which plays an important role in cell adhesion, tumor invasion and metastasis, was demonstrated to be overexpressed in CRPC cells and employed for the development of new targeted therapeutics (Deutscher et al., 2009; Laderach et al., 2013; Yang et al., 2014b; Zou et al., 2005). Peptide G3-C12 (the sequence ANTPCGPYTHDCPVKR) which specifically binds to the carbohydrate-recognition domain of Gal-3 was attached to HPMA-5-fluorouracil polymer-drug conjugate as a targeting moiety, P-(G3-C12)-Fu (Yang et al., 2012). It was showed that P-(G3-C12)-Fu exhibited higher tumor accumulation and significantly improved anti-tumor activity than free 5-Fu in nude mice bearing PC-3 tumor xenografts. They further demonstrated that Gal-3 overexpressed in two CRPC cells lines (PC-3 and DU145) could be effectively bind by G3-C12-modified HPMA copolymers by qPCR and competitive binding test (Yang et al., 2014b). Then they employed G3-C12-modified HPMA- doxorubicin conjugate (G3-C12-HPMA-Dox) for the treatment of CRPC as well as G3-C12- modified HPMA-Dox-Fu for the combination therapy against CRPC, exhibiting promising clinical potentials (Sun et al., 2015; Yang et al., 2015). However, it's worthy of noting that the potentials of G3-C12 have not been fully explored considering its specific targeting to CRPC cells and its success in tumor targeted drug delivery design.

In recent years, thioester bond has been employed for the design of novel prodrugs (Hu et al., 2014; Liu et al., 2008; Schoenmakers et al., 2004; Shen et al., 2010; Wang et al., 2013; Yue et al., 2012; Zhang et al., 2013; Zou et al., 2014), responsive polymers (Ali et al., 2006; Dan and Ghosh, 2013, 2014; Kim et al., 2012; Molla and Ghosh, 2012; Rieger et al., 2005; Rydholm et al., 2007; Vandenbergh et al., 2012; Wu et al., 2014), polymeric drug delivery nanocarriers (Chen et al., 2014; Dan et al., 2011; Jin et al., 2014b; Molla et al., 2014; Yang et al., 2014a), and gene delivery carriers (Oishi et al., 2005a,b, 2003; Tamur and Yui, 2013; Zhu et al., 2008) owing to its good biodegradability. Schoenmakers et al. (2004) firstly linked anticancer drug paclitaxel (PTX) with PEG via β and γ-thioester bond to fabricate drug-containing hydrogels. PTX could be released from the hydrogels with a half-life time of 4.2 ± 0.1 days for β-thioester bond and 14.0 ± 0.2 days for γ-thioester bond. With similar strategies, PEG-DTX conjugate, oligo polyethylene glycol- camptothecin (OEG-CPT) conjugate, OEG-2S-SN38 conjugate, and OEG-SN38 conjugate were developed (Liu et al., 2008; Shen et al., 2010; Wang et al., 2013; Zhang et al., 2013). Besides, Yue et al. (2012), Hu et al. (2014), and Zou et al. (2014) separately developed three different block copolymer-based polymeric PTX prodrugs. In addition to the design of responsive prodrugs, responsive nanocarriers were also developed for the delivery of anticancer drugs. Dan et al. (2011) fabricated a novel β-thiopropionate-containing surfactant via the Michael addition reaction between a hydrophobic thiol and hydrophilic acrylate derivative. The surfactant can aggregate into micellar nanoparticles and effectively encapsulate hydrophobic drugs. In the presence of acid, the surfactant degraded accompanied with the release of drugs. Jin et al. (2014b) prepared a novel amphiphilic ABA-type triblock copolymer poly(ethylene glycol)-*b*-poly(ethanedithiol-*alt*- nitrobenzyl)-*b*-poly(ethylene glycol) (PEG-*b*-PEDNB-*b*-PEG) by sequential Michael addition polymerization of



Scheme 1. Schematic illustration for the fabrication of castration-resistant prostate cancer (CRPC) targeting micellar nanoparticles via self-assembly of the amphiphilic brush-type polymers, P(OEGMA-*co*-G3-C12)-*g*-PCL. The micelles consist of PCL as hydrophobic core and hydrophilic corona of POEGMA and G3-C12. Hydrophobic anticancer drug, bufalin (BUF), was physically encapsulated into the hydrophobic cores of the micellar nanoparticles.