International Journal of Pharmaceutics 513 (2016) 612–627

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

One-step assembly of polymeric demethylcantharate prodrug/Akt1 shRNA complexes for enhanced cancer therapy

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ARTICLE INFO

Article history:
Received 2 June 2016
Received in revised form 22 September 2016
Accepted 24 September 2016
Available online 25 September 2016

Keywords:
Cancer therapy
Polymeric prodrug
Co-delivery
Akt1 shRNA
Demethylcantharate

ABSTRACT

This report demonstrated a one-step assembly for co-delivering chemotherapeutics and therapeutic nucleic acids, constructed by integrating drug molecules into a nucleic acid condensing polymeric prodrug through degradable linkages. Demethylcantharate was selected as the model drug and pre-modified by esterifying its two carboxylic groups with 2-hydroxyethyl acrylate. The synthesized demethylcantharate diacrylate was then used to polymerize with linear polyethyleneimine (PEI 423) through a one-step Michael-addition reaction. The obtained cationic polymeric demethylcantharate prodrug was used to pack Akt1 shRNA into complexes through a one-step assembly. The formed complexes could release the parent drug demethylcantharate and Akt1 shRNA through the hydrolysis of ester bonds. Cellular assays involving cell uptake, cytotoxicity, and cell migration indicated that demethylcantharate and Akt1 shRNA co-delivered in the present form significantly and synergistically suppress the growth and metastasis of three human cancer cells. This work suggests that incorporating drug molecules into a nucleic acid-packing cationic polymer as a polymeric prodrug in a degradable form is a highly convenient and efficient way to co-deliver drugs and nucleic acids for cancer therapy.

1. Introduction

To date, cancer combinatorial therapy involving chemotherapeutics and nucleic acids has been actively studied due to the extensively reported synergistic effects of the two different forms of active ingredients (Teo et al., 2015). The co-delivery of chemotherapeutics and nucleic acid is currently one of the most promising forms of cancer combinatorial therapy (Sun et al., 2011). Polymers have been extensively used for chemotherapeutic and nucleic acid co-delivery (Godsey et al., 2013; Khan et al., 2012). However, due to different physicochemical properties of chemotherapeutics and nucleic acids, loading them in polymer-based vectors usually needs elaborate and complicated processes. Firstly, chemotherapeutics, no matter their hydrophilic/hydrophobic properties, are loaded in the polymer-based vectors mainly through the methods of covalent conjugation or physical encapsulation. Normally, purification steps are needed to remove the unloaded drugs (Godsey et al., 2013; Khan et al., 2012). Secondly, negatively charged nucleic acids are usually packed into complexes by drug-loaded cationic polymers via electrostatic

Abbreviations: PEI, polyethyleneimine; DCAD, demethylcantharate diacrylate; DCA, demethylcantharate; poly(EI, *co*-DCA) poly(ethylenimine-co-demethylcantharate); NCTD, norcantharidin; HEA, 2-hydroxyethyl acrylate; DMEM, Dulbecco's Modified Eagle Medium; MTT, 3-(4 5-dimethylthiazol-2-yl)-2 5-diphenyltetrazolium bromide; Lipo 2000, Lipofectamine® 2000; FBS, fetal bovine serum; scr, scrambled shRNA encoded plasmid; ACTD, acryloyloxyethylnorcantharidin; DMAP, 4-(dimethylamino) pyridine; EDCI, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; GPC, gel permeation chromatography; PEG, poly (ethylene glycol); pDNA, plasmid DNA; TAE, tris-acetate; TEM, transmission electron microscopy; RLU, relative light units; GFP, green fluorescent protein; CLSM, confocal laser scanning microscope; RITC, Rhodamine B isothiocyanate; RITC-poly (EI-*co*-DCA), RITC labelled poly(EI-*co*-DCA); YOYO-1-Akt1 shRNA, YOYO-1 iodide labelled Akt1 shRNA; ECL, enhanced chemiluminescence; SD, standard deviation.

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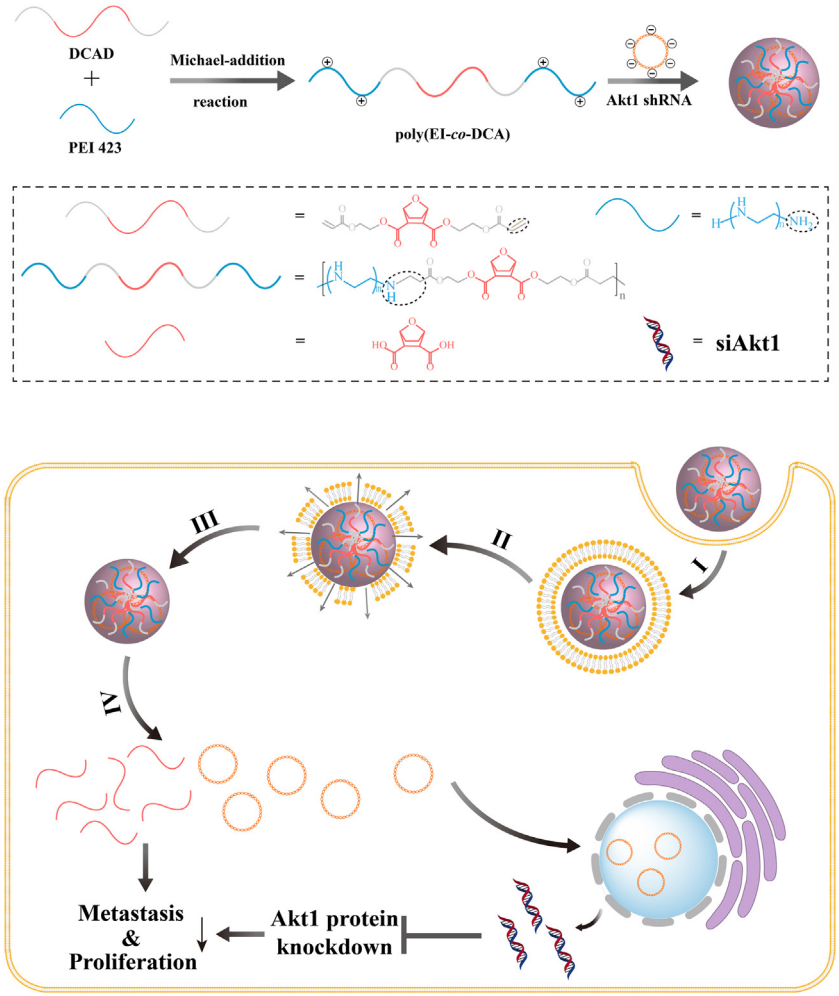
http://dx.doi.org/10.1016/j.ijpharm.2016.09.070
0378-5173/

condensation (Teo et al., 2015). A single-step assembly to load chemotherapeutics and nucleic acids into a structurally simple polymer-based delivery system is urgently needed to facilitate the searching of candidates for cancer combinatorial therapy.

Currently, a novel cationic polymeric drug has been developed and used for nucleic acid delivery, functioning both as a gene delivery vector and as an antagonist of the CXCR4 chemokine receptor (Li et al., 2012). Compared with the traditional polymer-based co-delivery vectors, this cationic polymeric drug-based co-delivery vector has many advantages, including (1) high drug loading efficiencies and contents (2) a simple synthesis method (a one-step Michael-addition reaction) (3) reduced contents of inert pharmaceutical materials (4) a one-step process for simultaneous loading of drugs and nucleic acids. Despite these advantages, one inevitable restriction for the cationic polymeric drug was that the polymer failed to be degraded into parent drugs, for which drugs used in the synthesis of cationic polymeric drug needed to afford minor structural modifications without compromising their therapeutic effects. However, the structural modifications of clinically available drugs usually lead to reduced therapeutic effects (Tong and Cheng, 2009). In this regard, few drugs could be used as candidates for the synthesis of cationic polymeric drugs. Therefore, new techniques must be developed to expand the scope of drug candidates for the synthesis of cationic polymeric drugs.

Herein, to solve the problems associated with cationic polymeric drugs, we designed and synthesized a novel degradable cationic polymeric prodrug, poly(ethylenimine-*co*-demethylcantharate) [poly(EI-*co*-DCA)] copolymers, which were synthesized by the Michael-addition reaction of low molecular weight linear PEI (PEI 423) with demethylcantharate diacrylate (DCAD). Demethylcantharidate (DCA), the active ingredient of clinically available sodium demethylcantharidate injection in China, is used as a routine anticancer drug to treat multiple malignancies including breast cancer, hepatoma, leukemia, abdominal cancer, *etc.* (Lu et al., 2012; Wang et al., 2006). By design, DCA was pre-modified with diacrylate groups through degradable bonds (here takes ester bonds as an example), which made sure the parent drug DCA could be successfully released from the cationic polymeric prodrug. Akt1, one of the important protein of serine-threonine kinase AKT pathway, determines the fate of the cancer cells when exposed to apoptotic stimuli such as chemotherapeutic drugs (Igney and Krammer, 2002), and previous studies have shown that using Akt1 short hairpin RNA (Akt1 shRNA) to knock down the expression of Akt1 protein is an effective way for cancer therapy (Jiang et al.,



Scheme 1. Transport Akt1 shRNA and DCA to cancer cells by poly(EI-*co*-DCA) copolymer/Akt1 shRNA complexes for synergistic therapy. (I) Endocytosis of poly(EI-*co*-DCA) copolymer/Akt1 shRNA complexes by cancer cells; (II) Endosomal bursting caused by proton sponge effect of poly(EI-*co*-DCA) copolymer/Akt1 shRNA complexes; (III) Endosomal escape of poly(EI-*co*-DCA) copolymer/Akt1 shRNA complexes; IV: Hydrolysis-induced degradation of poly(EI-*co*-DCA) copolymer/Akt1 shRNA complexes and the release of DCA and Akt1 shRNA.