



Endolysosomal environment-responsive photodynamic nanocarrier to enhance cytosolic drug delivery via photosensitizer-mediated membrane disruption

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ARTICLE INFO

Article history:

Received 1 July 2013

Accepted 14 August 2013

Available online 2 September 2013

Keywords:

Photosensitizer

FRET

Enzyme-responsive material

Endosomal escape

Cytosolic drug delivery

ABSTRACT

The endolysosome is a major barrier for the effective intracellular delivery by conventional nanocarriers. Herein, we demonstrate that endolysosome environment-responsive photodynamic nanocarriers (EPNs) are capable of encapsulation of the hydrophobic drug paclitaxel (PTX) and photosensitizer (PS)-mediated ELB disruption for effective cancer therapy. EPNs were self-assembled from PS (chlorin e6, Ce6) or Black Hole Quencher-3 (BHQ3) conjugated covalently to polypeptide-based amphiphilic copolymers [mono-methoxy polyethylene glycol-block-poly(β -benzyl-L-aspartic acid), mPEG-pBLA]. EPNs have a spherical shape and a unimodal size distribution below 100 nm. Photoquenching of the EPNs was dependent on the molar ratio of mPEG-pBLA-BHQ3/mPEG-pBLA-Ce6. However, in the presence of the endolysosomal enzyme (e.g., esterase), the benzyl ester bond is cleaved which leads to the structural collapse of EPNs, thus triggering drug release and restoring photoactivity. Live cell imaging studies demonstrated that PS-mediated lipid peroxidation significantly increased the ability of model drug (*i.e.*, Nile red) to overcome the ELB. In comparison with PTX treatment alone, the combined treatment of PTX encapsulated EPNs with laser irradiation synergistically induced the death of HeLa and drug-resistant HCT-8 cells *in vitro*, and suppressed CT-26 tumor growth *in vivo*. These results suggest that this approach is a promising platform for cancer treatment. Furthermore, this EPN system offers significant potential for effective cytosolic delivery of chemical and biological therapeutics.

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1. Introduction

In recent decades, drug carrier systems based on nanoparticles (NPs) have exhibited promise in overcoming the lack of selectivity in chemotherapy. The enhanced permeability and retention (EPR) effect exhibited by these systems can help to avoid damaging healthy tissue and improve cancer treatment [1,2]. However, conventional NP drug delivery systems have achieved rather limited success to date, owing primarily to the biological barriers present under *in vivo* conditions [3–5]. Among them, endosomal sequestration is one of the particularly important barriers to the delivery of chemotherapeutic agents. Because chemotherapy drugs trapped in endosomes are typically trafficked into lysosomes and degraded [6,7]. In this regard, a variety of polymers or oligomers bearing functional groups that undergo reversible protonation–deprotonation cycles have been utilized to overcome the endolysosomal

barrier (ELB) [8–10]. However, although protonatable polymers show proton buffering in a pH range of 5–7, no enhancement of cellular internalization noticeable due to a lack of ELB penetration [11–13]. This issue suggests a need for endosomolytic agents or novel methodology to enable more effective cytosolic drug delivery.

Photochemical internalization (PCI), an innovative approach to extended photodynamic therapy (PDT), has been utilized more recently for light-induced cytoplasmic drug delivery. The mechanism of PCI is based on the breakdown of the ELB membrane by reactive oxygen-induced lipid peroxidation [14,15]. The attraction of PCI is that the light dose required to induce PCI is much lower than that required to induce PDT [16,17]. Therefore, a deeper treatment effect can be achieved with PCI than with PDT for the same delivered light dose. Our group has previously reported a PCI system for improved cellular delivery of nucleic acids [18]. However, PCI may cause phototoxicity due to poor target selectivity. This can lead to side effects, such as blood cell and vascular damage, which limit its potential for clinical application [19,20]. It is therefore necessary to develop next-generation NP-based drug carrier systems, such as stimuli-responsive on/off systems, with the

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goal of achieving photo-activity control. Such systems are expected to improve therapeutic effects dramatically in tumors and effectively reduce systematic toxicity.

In this study, we demonstrate an endolysosome environment-responsive photodynamic nanocarriers (EPNs) as intracellular enzyme-activatable ELB disruptors for effective cancer therapy. In designing the structure, an enzyme-responsive “on/off” module was introduced into a polymeric nanocarrier to turn “off” both the photo-activity of PS and drug release during blood circulation. The EPNs would then turn “on” under enzyme-rich conditions, such as those found in the endolysosomes of cancer cells, through an enzyme-mediated structural alteration that would change its character from hydrophobic to hydrophilic (Scheme 1a). When subsequently exposed to light in a particular section of the tumor, the PS moiety would cause collapse of the ELB and release the encapsulated drug into the cytosol (Scheme 1b). We therefore hypothesized that an EPN loaded with paclitaxel (PTX) would show significant enhancement of chemotherapeutic efficiency in cancer cells via ELB disruption and induced release of the drug into the cytosol.

2. Materials and methods

2.1. Materials

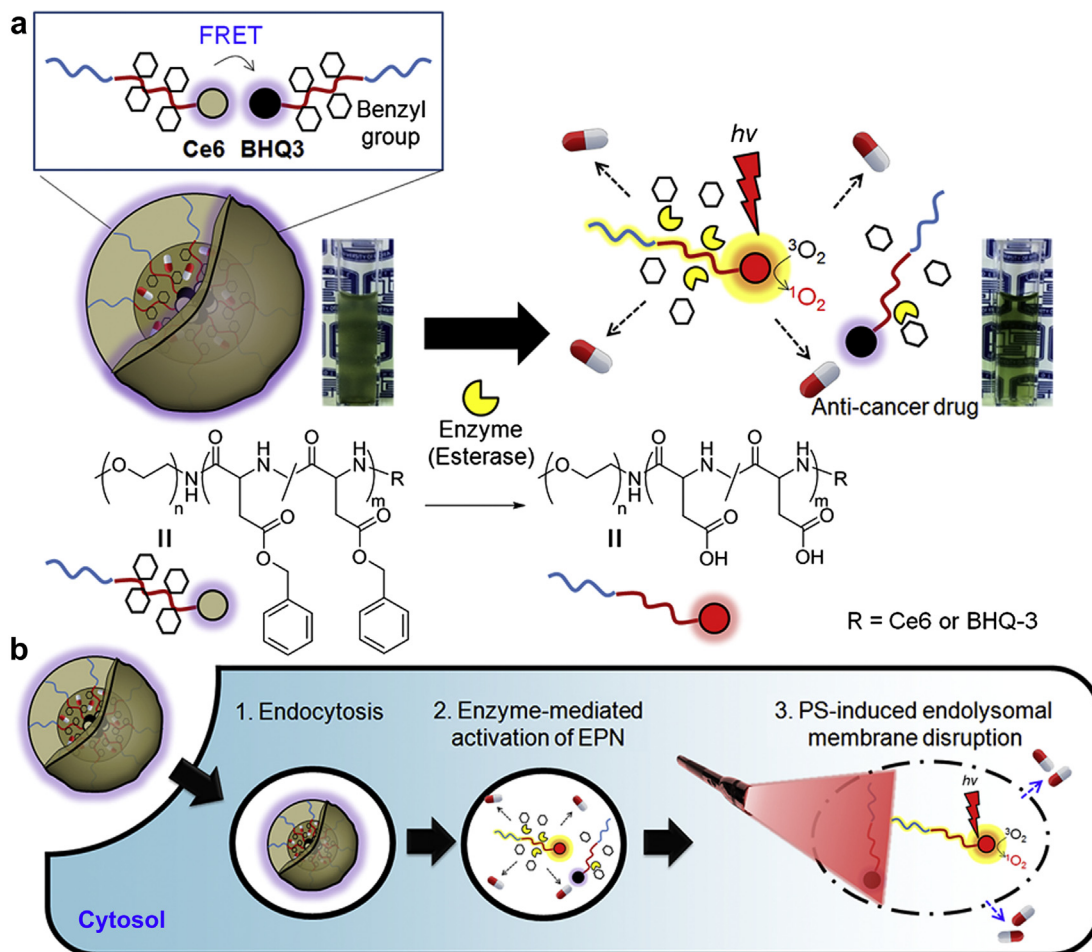
β -Benzyl-L-aspartic acid (BLA), bis-(trichloromethyl)-carbonate (triphosgene), *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), dichloromethane (DCM), 1,3-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), 3-(4,5-

dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), esterase from porcine liver, Hoechst 33342 and Nile red (NR) were purchased from Sigma–Aldrich. Tetrahydrofuran (THF) was purchased Wako. Methoxy polyethylene glycol amine (mPEG-amine, MW 5000) was purchased from Sunbio, Inc. (Anyang, Korea). Chlorin e6 (Ce6) was purchased from Frontier Scientific, Inc. (UT, USA). Black Hole Quencher-3 carboxylic acid (BHQ3) was purchased from Biosearch Technologies Inc. (CA, USA). The 4',6-diamidino-2-phenylindole (DAPI), LysoTracker Green DND-26 were purchased from Molecular Probes, Inc. (OR, USA). The TBARS assay kit was purchased from Cayman Chemical Co. (MI, USA). The dialysis membranes were obtained from Spectrum Laboratories Inc. (CA, USA). RPMI-1640 medium, DMEM medium, fetal bovine serum (FBS), antibiotics (penicillin/streptomycin), and Dulbecco's phosphate buffer saline (DPBS) were obtained from Gibco BRL (Invitrogen Corp., CA, USA). Singlet Oxygen Sensor Green (SOSG) was purchased from Molecular Probes, Inc. (OR, USA). Paclitaxel (PTX) was purchased from Samyang Genex corp. (Seoul, Korea). All of the other chemicals and solvents were analytical grade.

2.2. The synthesis of mPEG-pBLA

β -Benzyl-L-aspartic acid *N*-carboxyanhydride (BLA-NCA) was synthesized by the Fuchs–Farthing method using triphosgene. Briefly, β -Benzyl-L-aspartic acid (3 g, 13.44 mmol) was suspended in THF (50 mL) containing triphosgene (3 g, 10.11 mmol) and stirred at 60 °C for 2 h. The crude mixture was filtered twice. The product was precipitated by the addition of hexane (900 mL) and collected by filtration and drying under vacuum to obtain BLA-NCA as a white powder.

mPEG-pBLA was synthesized by ring-opening polymerization of BLA-NCA initiated by the terminal amino group of mPEG-amine. The mPEG-amine (1.15 g, 0.23 mmol) was dissolved in DCM (50 mL). A solution of BLA-NCA (2.87 g, 11.52 mmol) in DMF (20 mL) was added to the solution of mPEG-amine and the reaction mixture was stirred for 48 h at room temperature. The crude mixture was filtered and concentrated on a rotary evaporator under vacuum at 60 °C for 30 min. The resulting solution was dialyzed against distilled water using a dialysis membrane (Spectra/Por; mol. wt. cutoff = 3500) for 2 days. Lyophilization afforded



Scheme 1. Schematic conceptual representation of a) endolysosome environment-responsive photodynamic nanocarrier (EPN) with images of EPN dissolved in aqueous solution (before and after enzyme treatment) and b) PS-mediated ELB disruption; see text for details.

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