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Polyelectrolyte nanocomplex formation of heparin-photosensitizer conjugate with polymeric scavenger for photodynamic therapy

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ABSTRACT

A polyelectrolyte nanocomplex was prepared via the ionic interaction between the anionic heparin-pheophorbide a (HPhA) conjugate, which served as a water-soluble polysaccharide photosensitizer (PS), and the cationic polyethylenimine (PEI)-β-carotene (PCAR) conjugate, which served as a polymeric scavenger. This nanocomplex was designed to improve the water solubility and tumor specificity of PhA and to only release singlet oxygen at the tumor cell. A stable 150 nm-sized nanocomplex could be formed in the weight ratio range (PCAR/HPhA) of 0.3–0.5 in an aqueous environment. The PCAR scavenger significantly diminished the generation of active singlet oxygen from HPhA in a buffer solution. Singlet oxygen scavenging activity was lost only when HPhA and PCAR were separated from each other due to the dissociation of the complex nanostructures. It was confirmed that HPhA itself has neither colloidal properties nor a decrease in its ability to produce singlet oxygen. At the same time, the HPhA/PEI complex produced singlet oxygen in response to light. In a cell culture system, the cytotoxicity of the HPhA/PCAR nanocomplex toward cancer cells was greatly enhanced due to the efficient generation of singlet oxygen under light irradiation; this finding implies that the scavenging activity of PCAR can be restricted to intracellular environments. These results suggest that the HPhA/PCAR nanocomplex could provide a new activatable PS platform that facilitates more accurate and reliable photodynamic therapy (PDT) with site-specific and controllable production of singlet oxygen to be used for the treatment of cancer.

1. Introduction

Photodynamic therapy (PDT) has been widely applied in the clinic as a non-invasive therapeutic regimen to treat cancer (Dolmans, Fukumura, & Jain, 2003; Juarranz, Jaen, Sanz-Rodriguez, Cuevas, & Gonzalez, 2008; Wilson, 2002). In PDT, photosensitizing agents, or photosensitizers (PSs), are typically administered via intravenous routes; PSs are supposed to accumulate at tumor sites (Hamblin & Mróz, 2008; MacDonald & Dougherty, 2001). Upon light irradiation with a specific wavelength, PSs activate and generate highly reactive singlet oxygen from nearby oxygen molecules. This process results in tumor cell death and proximal tissue necrosis (Fernandez, Bilgin, & Grossweiner, 1997; Vrouenraets, Visser, Snow, & van Dongen, 2003). Most clinically available PSs (e.g., porphyrin and phthalocyanine derivatives) have drawbacks, such as poor water solubility and low tumor cell selectivity (Detty, Gibson, & Wagner, 2004; Konan, Gurny, & Allemann, 2002). The lack of tumor specificity often leads to unexpected damage to normal tissues; skin photosensitivity can also occur, even to some indoor light sources (Detty et al., 2004; Vrouenraets et al., 2003).

For successful PDT via PS delivery to tumor cells, the following two requirements should be fulfilled: the controllable generation of singlet oxygen and the tumor-selective accumulation of PSs (Li, Nurunnabi, Nafiujjaman, Jeong, Lee, & Huh, 2014; Li, Nurunnabi, Nafiujjaman, Lee, & Huh, 2013; Lovell, Liu, Chen, & Zheng, 2010; Park, Park, & Na, 2011; Verhille et al., 2010). During PDT, photodamage to target cells is highly dependent on the level of singlet oxygen generation stimulated by light irradiation. By controlling the generation of singlet oxygen, the level of singlet oxygen in the bloodstream is minimized, and the availability of active singlet oxygen within tumor tissue is maximized. The use of activatable PSs can satisfy this first requirement. Generally, activatable PSs generate singlet oxygen only within tumor tissues and remain inert during systemic circulation. For example, activatable PSs for targeted PDT can be constructed by the chemical conjugation of PS molecules

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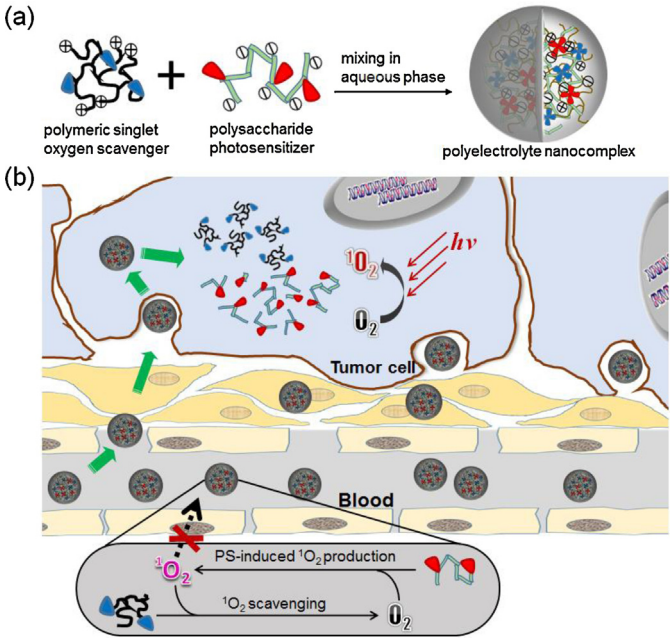

Fig. 1. Schematic concept of a proposed polyelectrolyte nanocomplex with tumor accumulation capacity via the enhanced permeability and retention effect (EPR) and the controllable production of singlet oxygen. (a) The nanocomplex designs to be prepared by ionic interactions between an anionic polysaccharide PS and a polymeric singlet oxygen scavenger. (b) The nanocomplexes do not produce cytotoxic reactive oxygen species during blood circulation because the polymeric scavenger depletes the generated singlet oxygen; therefore, the proposed nanocomplex is non-phototoxic during blood circulation and reduces the side effects observed with conventional PDT. When the nanocomplexes are internalized by cancer cells, the enzymatic degradation of polysaccharide chains and ionic exchange interactions induced by intracellular ions trigger the dissociation of the nanocomplexes. The PS and scavenger become spatially isolated, thereby restricting the scavenging effects and restoring the phototoxicity of the PS.

to a certain type of activation controller via stimuli-sensitive or cleavable linkers. Such linkers can be cleaved or degraded by physicochemical or biological stimuli that are specific to or over-expressed only within tumor tissue.

To date, activatable PSs have been constructed as low-molecular-weight systems such as PS–PS conjugates (McCarthy & Weissleder, 2007), PS-quencher conjugates (Lovell et al., 2009) or PS-singlet oxygen scavenger conjugates (Chen et al., 2007). Low-molecular-weight systems have limitations, such as the rapid elimination of conjugates from the body and the development of multiple drug resistance; these issues can result in short plasma half-life and low bioavailability (Master, Livingston, & Sen Gupta, 2013). Furthermore, the preparation of an activatable PS system often involves multiple synthetic procedures and purification steps. This poses a practical challenge to their application (Verhille et al., 2010).

Before the switching-on mechanism of the activatable PS in this study is presented, its physical structure and the theory of tumor targeting should be explained. We designed a polyelectrolyte nanocomplex platform produced by the ionic interaction between a water-soluble polysaccharide PS and a polymeric scavenger (Fig. 1(a)). Unlike conventional low-molecular-weight activatable PSs, the nanocomplex system is expected to have a prolonged blood circulation time due to its nanometer size and biocompatible particle surface. An increase in the selective accumulation of PSs at tumor sites is expected as a consequence of the enhanced permeability and retention (EPR) effect (Maeda, 2001; Nehoff, Parayath, Domanovitch, Taurin, & Greish, 2014); such accumulation is required for successful PDT. As shown in Fig. 1(b), the nanocomplex PS system has the potential to positively modulate pharmacokinetic and toxicological properties and the pharmacodynamic end-point of loaded PSs (Park et al., 2011).

In this study, an anionic heparin-pheophorbide a (HPhA) conjugate was synthesized as a water-soluble polysaccharide PS, and a cationic polyethylenimine (PEI)-β-carotene conjugate (PCAR) was synthesized as a polymeric scavenger. These components were utilized to produce nanocomplex PSs by the electrostatic interaction between oppositely charged polyelectrolytes. PhA has very low solubility in water; therefore, its practical utilization as a pharmaceutical agent has been limited (Robert, 1986). To overcome this problem, we synthesized a water-soluble PhA by conjugating it to heparin. Heparin, a negatively charged polysaccharide, has been used in the pharmaceutical and biomedical fields due to its excellent water solubility, biocompatibility, and biodegradability (Linhardt, 1991). CAR, a singlet oxygen scavenger, was introduced to control the availability of singlet oxygen generated from HPhA molecules. However, CAR is a small, hydrophobic molecule. It is therefore difficult to directly combine CAR with water-soluble HPhA conjugates. We conjugated CAR molecules to water-soluble branched PEI (bPEI) to create a positively charged macromolecular scavenger to be electrostatically complexed with HPhA. One advantage of the proposed nanocomplex over conventional activatable PSs is that the water-soluble form of this colloid-stable, nanometer-sized PS can be simply prepared by mixing two aqueous solutions of HPhA and PCAR without the use of an organic solvent or a complicated preparation process.

The HPhA/PCAR nanocomplex has tumor-site specificity and is easily prepared; in addition, it is designed to exhibit switchable phototoxicity. We previously described a PhA/CAR dual-loaded micellar system in which singlet oxygen production for PDT can be controlled (Li, Cho, Yoon, Kang, & Huh, 2014). In micelles, the antioxidant agent CAR significantly diminished singlet oxygen quantity through direct scavenging. Thus, the HPhA/PCAR nanocomplex is expected to restrict the phototoxicity of PhA and minimize the complications observed in traditional PDT. It is hypothesized that the intravenously administered nanocomplex is localized to tumor tissue and endocytosed by tumor cells. After internalization, HPhA and PCAR might dissociate via ionic exchange; heparin may also be degraded enzymatically. We predict that the spatial separation of PhA and CAR within cells can prevent the CAR-mediated scavenging of the singlet oxygen generated by the PhA, resulting in the switching-on of photodynamic therapy.

As a proof-of-principle study, this work aims at establishing the preparation conditions for the formation of an HPhA/PCAR nanocomplex with appropriate particle size and stability in a physiological environment. This nanocomplex was investigated with regard to its physicochemical characteristics, photoactivity, and in vitro PDT efficacy in tumor cell culture systems.

2. Materials and methods

2.1. Materials

Unfractionated heparin (MW 12,000 Da) was obtained from Mediplex Co. (Korea). Pheophorbide a (PhA) was purchased from Frontier Scientific Inc. (Logan, UT, USA). Branched polyethylenimine (bPEI) (MW 25,000 Da) was obtained from Sigma–Aldrich (St. Louis, MO, USA). *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl), trans-β-apo-8′-carotenal, sodium triacetoxyborohydride (STAB), 1,2-dichloroethane (DCE), acetic acid (AcOH), dimethyl sulfoxide (DMSO), 9,10-dimethylanthracene (DMA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), RPMI 1640 medium, Ca^{2+}- and Mg^{2+}-free Dulbecco's