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Short communication

Combretastatin A4/poly(*L*-glutamic acid)-graft-PEG conjugates self-assembled to nanoparticles

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ABSTRACT

Combretastatin A4 (CA4) possesses varying ability to cause vascular disruption in tumors, while the short half-life, low water solubility and deactivation of many CA4 analogs during storage limited its antitumor efficacy and drug stability. A novel macromolecular conjugate of CA4 (CA4-PL) was synthesized by covalent bonding of CA4 onto poly(*L*-glutamic acid)-graft-polyethylene glycol (PLG-g-PEG) via Yamaguchi reaction. The obtained CA4-PL was characterized by ^{1}H NMR, GPC, and UV methods, and the properties of the nanoparticles composed of CA4-PL, including critical aggregation concentration, size and size distribution, and morphology, were investigated. CA4-PL can self-assemble to form micelle-like nanoparticles of 80~120 nm in diameter, which may have potential to improve the blood circulation period as well as the targetability of CA4, and find applications to treat various tumors when combined with traditional chemotherapy or radio therapy.

1. Introduction

Combretastatins are a class of natural phenols that are present in the bark of *Combretum caffrum*, commonly known as South African bush willow, and a variety of synthesis routes of the combretastatin skeleton are available [1]. Members of the combretastatin family possess ability to cause vascular disruption in tumors via binding to the β-subunit (known as the colchicine site) of tubulin, inhibiting tubulin polymerization, and thus preventing the synthesis of microtubules. CA4 is an effective antimitotic agent possessing potent cytotoxicity against

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a panel of cancer cells, including multi-drug resistant cancer cell lines, and previous studies also indicated that CA4 prodrug induced selective antivascular effects against tumor-associated endothelium [2]. In recent years, CA4 and its analogs were evaluated in clinical trials, including the phase II/III trials of phosphate prodrug of CA4 to treat neuroendocrine tumors or ovarian cancer as sponsored by OXiGENE Company. A phase I pharmacokinetic study revealed rapid dephosphorylation of the parent compound to CA4, with a short plasma half-life of only approximately 30 min [3].

The short half-life, as well as the low water solubility and deactivation of many CA4 analogs during storage greatly limited their antitumor efficacy and drug stability. Various CA4 analogous with increased water solubility, or cis-restriction property were synthesized for improving their bioavailability and efficiency [4]. Conjugation of drugs onto polymeric molecules to form macromolecular conjugates is a promising approach instead of formulating drugs with solubilizer(s) to circumvent the inadequate solubility of many anticancer drugs [5]. Pegylated polymeric nanoparticles or macromolecular conjugates also bear potentials of improving the stability and blood circulation period, and altering their *in vivo* distribution compared to the parent drugs.

Here, we reported a novel CA4 macromolecular conjugate (CA4-PL) by chemically linking CA4 molecule to the pegylated poly(glutamic acid) copolymer. As an amphiphilic macromolecule, CA4-PLs can self-assemble to form nanosized particles in aqueous media. Long circulation and improved tumor targeting depending on the pegylation modification and the well-known EPR effect were expected for such nanoparticles.

2. Materials and methods

2.1. Materials

CA4 was purchased from Great Forest Biomedical Ltd. (Hangzhou, China). Poly(glutamic acid) (PLG) was received from Changchun Institute of Applied Chemistry (Jilin, China). Monomethoxy PEG5000 (mPEG) was purchased from Sigma-Aldrich (Shanghai, China). 2,4,6-trichlorobenzoyl chloride (TCBC) was purchased from Heowns Biochemical Technology Co., Ltd. (Tianjin, China). Triethylamine (NEt_3), *N,N*-dimethyl formamide (DMF), ether, dichloromethane, deuterated water, and deuterated sodium hydroxide were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). DMF was dried with calcium hydride and distilled at reduced pressure before use. *N,N'*-Diisopropylcarbodiimide (DIC) and N-(4-Pyridyl) dimethylamine (DMAP) were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Sodium chloride (NaCl), sodium dihydrogen phosphate (NaH_2PO_4) and sodium hydrogen phosphate (Na_2HPO_4) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Synthesis of PLG-g-PEG

PLG-g-PEG was synthesized according to previously reported procedure [6]. Briefly, PLG was prepared by the ring-opening polymerization of BLG-NCA using n-hexylamine as the initiator at monomer/initiator molar ratio of 160. PLG and dried mPEG were the dissolved in anhydrous DMF by heating at 40 °C for 2 h. After the temperature was cooled down to 25 °C, DIC and DMAP were added in succession. After stirring at 25 °C for 2 d, the reaction mixture was precipitated by pouring into excess volume of ether and washed twice with ether. The precipitate was dried under vacuum and re-dissolved in DMF, placed in dialysis tube (MWCO 7000, Greenbird Technology Development Co. Ltd, Shanghai, China) and dialyzed against distilled water, and then freeze-dried to produce the PLG-g-PEG (white powder).

2.3. Synthesis of CA4-PL

CA4-PL was synthesized by conjugating CA4 to PLG-g-PEG via Yamaguchi reaction with certain modification (see Scheme 1). Briefly, 600 mg of PLG-g-PEG was placed into dry flask and dissolved in 20 ml of dry DMF, and then 161 mg of NEt_3 dissolved in 1 ml of DMF and 390 mg of TCBC dissolved in 1 ml of DMF were added and mixed uniformly. 316 mg of CA4 dissolved in 2 ml of DMF, and 122.7 mg of DMAP dissolved in 2 ml of DMF were then added. The reaction was continued for 12 h under constant stirring. The reaction mixture was poured into excess volume of ether, centrifuged at 8000 rpm, discarded the supernatant. After drying under vacuum, the precipitate was re-dissolved in DMF and diluted with distilled water, ultra filtrated to remove DMF and impurities. The solution was then filtered and freeze-dried to produce the solid powder CA4-PL (yield: 60%).

2.4. Characterizations to CA4-PL

^{1}H NMR spectra were recorded on an AV 400 NMR Spectrometer (Bruker, Germany) in sodium deuteroxide/deuterium oxide ($NaOD/D_2O$) solution. GPC measurements were conducted on a GPC system (Ultrahydrogel Linear column, 1515 HPLC pump with 2414 Refractive Index Detector, Waters, USA) using phosphate buffer (0.2 M, pH 7.4) as the eluent (flow rate: 1 ml/min, 25 °C), and polyethylene glycol was used as standards. UV-Vis spectra of samples dissolved in DMF was scanned on a UV-5100 Spectrophotometer (Wanyee Science and Technology Co., Ltd, Anhui, China) at the wavelength range between 200 nm and 400 nm using DMF as the blank control.

2.5. Properties of the self-assembled nanoparticles

Critical aggregation concentration (*cac*) of CA4-PL was determined by fluorescence spectroscopy method with pyrene as the probe using a LS50B Luminescence Spectrometer (Perkin-Elmer, USA) with emission wavelength of 392 nm. The excitation fluorescence at 339 and 335 nm was monitored. Dynamic light scattering (DLS) measurements were performed on a Wyatt QELS instrument with a vertically polarized He-Ne laser (Wyatt Technology, USA) at 90° collecting optics. Zeta-potential was measured with a Zeta Potential/BI-90 Plus Particle Size Analyzer (Brookhaven, USA) at ambient temperature. A drop of the nanoparticle solution (about 0.1 mg/ml CA4-PL in water) was deposited onto a 230 mesh copper grid coated with carbon and allowed to dry in air at 25 °C before observation. TEM

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