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Acetal-linked polymeric prodrug micelles for enhanced curcumin delivery

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ABSTRACT

On-demand curcumin delivery via stimuli-responsive micellar nanocarriers holds promise for addressing its solubility and stability problem. Polymer-curcumin prodrug conjugate micelle is one of such nanosystems. The diversity of linker and conjugation chemistry enabled the generation and optimization of different curcumin micelles with tunable stimuli-responsiveness and delivery efficiency. The aim of the current work was to generate and assess acetal-linked polymeric micelles to enrich the pH-responsive curcumin delivery platforms. Curcumin was slightly modified prior to conjugating to amphiphilic methoxy poly(ethylene glycol)-poly(lactic acid) (mPEG-PLA) copolymer via an acetal bond, whereas an ester bond-linked conjugate was used as the control. The acetal-containing micelles showed a hydrodynamic diameter of 91.1 ± 2.9 (nm) and the accompanying core size of 63.5 ± 7.1 (nm) with a zeta potential of -10.9 ± 0.7 (mV). Both control and pH-labile micelles displayed similar critical micelle concentration at 1.6 μM. The acetal-containing nanocarriers exhibited a pH-dependent drug release behavior, which was faster at lower pH values. The cytotoxicity study in HepG2 cells revealed a significantly lower IC_{50} at 51.7 ± 9.0 (μM) for acetal-linked micelles in contrast to the control at 103.0 ± 17.8 (μM), but the polymer residue showed no cytotoxicity upon drug release. The acetal-linked micellar nanocarrier could be a useful addition to the spectrum of currently available stimuli-responsive curcumin nanoformulations.

1. Introduction

As a natural pleiotropic agent for cancer management, curcumin has been capable of regulating multiple cell signaling pathways, exhibiting a diverse range of beneficial biological functions, e.g. anti-inflammatory and anti-oxidant effects [1]. The uniqueness of curcumin for cancer chemotherapy is that it can act as both an anti-cancer drug and a chemosensitizer for reversing multidrug resistance [2]. In addition, as a well-known food additive, its safety profile is favorable for diminishing off-target toxicity since the majority of systemically administrated chemotherapeutics distribute in the non-target healthy organs/tissues [3]. However, its poor bioavailability has been a big concern due to the low absorption, fast metabolism and rapid elimination, which is associated with curcumin's low aqueous solubility (<1 μg/mL) and chemical instability [4].

The exploitation of various nanocarriers for curcumin delivery has been previously investigated to overcome the above limitations, including both lipid- and polymer-based nanosystems as well as cyclodextrin and peptide carriers [5–7]. In these reports, curcumin was primarily loaded via three approaches: chemical conjugation, physical encapsulation, and the combination of chemical and physical modes [8–10]. The non-covalent loading mode is simple, but it is often associated with premature drug release and insufficient curcumin encapsulation, which is the case for impotent curcumin. The covalent means can address these bottlenecks, but the parent drug modification is often necessary prior to conjugation and the synthetic procedures are tedious. The combinational load-

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ing mode is an effective method to manipulate nanocarrier stability, drug loading capacity and release kinetics [11].

Irrespective of the loading method, stimuli-responsive nanocarriers as programmed nanomedicine are appealing and capable of realizing on-demand curcumin delivery [12]. Tailored design of such nanosystems has relied on skilfully harness the cellular microenvironments e.g. pH, redox potential, enzyme, and other specific variations that naturally exist in vivo [13]. A diverse range of curcumin nanocarriers have been reported [14–17], among which redox potential and pH are two important triggers on account of the cellular microenvironment. In contrast to the redox-responsive system, pH-responsive nanocarrier often initiates a more rapid drug release and therefore early onset of pharmacological action. There are various types of pH-labile linkers including ester, hydrazone, hydrazide, imine, oxime, acetal, ketal and methyl maleate [18]. Despite the abundance of designing choices for pH-responsive nanocarriers, the linker selection determines the ease of drug conjugation, stimuli-responsiveness, residue toxicity and hence the delivery performance. For example, the hydrazone-containing nanosystem has been fashionable due to the simple synthetic process and ambient reaction condition, but such nanocarrier has posed the toxicity issue of cationic polymer residue upon cargo release [19]. Acetal as an acid-cleavable linker offers the benefit of stability with respect to many reducing and oxidizing chemicals, which can facilitate the synthesis of prodrug conjugates [20]. Moreover, all the degradation products are neutral without the hassle of toxicity associated with cationic excipients.

The aim of the current work was to design, synthesize and in vitro evaluate the acetal-linked polymer-curcumin prodrug conjugate micelles to enrich the curcumin nanoformulation pool (Fig. 1). Biodegradable methoxy poly(ethylene glycol)-poly(lactic acid), i.e. mPEG-PLA was employed as the biocompatible block copolymer. Curcumin was conjugated to the monovalent polymer via a pH-labile acetal linker. Likewise, the drug was also linked to the polymer via the ester bond, forming a pH-insensitive control. Upon endocytosis and drug release, the remaining of polymer residue is neutral that is presumed to be non-toxic. As the focus of the current work was the effect of linker chemistry on curcumin delivery in polymeric conjugate micellar systems, the mPEG-PLA micelles with drug being physically loaded were not investigated. In addition, although the polycurcumin had been reported as the carrier-free nanoparticulate delivery systems with high drug loading, it was not employed as the control in the current work because the focus of the current study is the linker other than the multivalency and carrier-free approaches [21,22].

2. Experimental

2.1. Materials

Anhydrous diethyl ether, triethylamine, hydrochloric acid, and anhydrous sodium sulfate were purchased from Jiangtian Chemicals (Tianjin, China). Tetrahydrofuran, succinic anhydride, dimethylformamide (DMF), curcumin, potassium iodide (KI), anhydrous potassium carbonate, methanol, citric acid, disodium hydrogen phosphate, acetone, poly(ethylene glycol) (PEG, 400 Da), *p*-toluenesulfonic acid (P-TSA) were obtained from Guangfu Fine Chemical Research Institute (Tianjin, China). Methoxy poly(ethylene glycol) (mPEG, 2000 Da), ethylene oxygen ethanol (EGVE), 4′,6-diamidino-2-phenylindole (DAPI) were from Sigma–Aldrich (Beijing, China). Stannous octoate, 4-dimethylaminopyridine (DMAP), pyrene were from Aladdin Industrial Corporation (Shanghai, China). Dulbecco's modification of eagle's medium (DMEM), fetal bovine serum were purchased from Gibco (NY, U.S.A). D, L-lactic acid was from Daigang Biomaterial Co., Ltd (Jinan, China). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) was from Medpep Co., Ltd (Shanghai, China). Molecule sieves was from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). 3-bromo-1-propanol was from J&K Co., Ltd. (Beijing, China). Sodium dodecyl sulphate (SDS) was from Bodi Chemical Co., Ltd. (Tianjin, China). Phosphate buffer (PBS) was from Airui Biological Technology Co., Ltd. (Shanghai, China). Trypsin was from Fangcheng Biological Technology (Shanghai, China). Cell Counting Kit-8 (CCK-8) was from Baili Biological Technology Co., Ltd. (Shanghai, China). Paraformaldehyde was from Solarbio Co., Ltd. (Beijing, China).

2.2. Synthesis of curcumin derivative

Because curcumin contains two phenol groups exhibiting low reaction activity, we designed one curcumin derivative to ease its conjugation with polymer. The hydroxyl-terminated curcumin derivative (Cur—OH) was prepared as follows (Scheme 1). Curcumin 2 g (5.43 mmol), anhydrous potassium carbonate 1.5 g (10.87 mmol), KI 0.092 g (0.543 mmol) and 3-bromo-1-propanol (590 μL, 6.53 mmol) were mixed in a round-bottom flask containing 20 mL DMF. This mixture was maintained at 25 °C for 24 h. After DMF removal by a rotary evaporator, the solid residue product in the round-bottom flask was dissolved with ethyl acetate followed by successive extraction with hydrochloride acid (HCl) solution (0.1 M) and water successively. The water residue in organic phase was removed using sodium sulphate (anhydrous) and then ethyl acetate was evaporated to get a solid product that was further purified by silica gel column chromatography to get the curcumin derivative Cur—OH (yield: 36.2%). ^{1}H NMR (400 M, DMSO-d_6), δ[ppm]: δ1.88 (15, m, 2H); δ3.55 (16, t, 2H); δ3.83 (1, s, 3H); δ3.84 (11, s, 3H); δ4.07 (14, t, 2H); δ6.08 (7, 1H); δ6.75–6.84 (6/8, m, 2H); δ7.01–7.35 (2/3/4/10/12/13, m, 6H); δ7.55–7.58 (5/9, q, 2H) (Supporting Information/SI, Fig. S1).

2.3. Synthesis of mPEG-PLA-EGVE

The synthesis of methoxy poly(ethylene glycol)-poly(lactic acid) (mPEG-PLA) utilized the typical ring-opening polymerization of D, L-lactide; the production of a carboxyl terminal (mPEG-PLA-COOH) was achieved with the presence of succinic anhydride using a previously published method [23]. To introduce an acetal linker between polymer and curcumin, mPEG-PLA-COOH was firstly modified by EGVE (Scheme 1). In brief, mPEG-PLA-COOH (1 g, 0.267 mmol), DMAP (0.0976 g, 0.800 mmol), EDC·HCl (0.144 g, 0.800 mmol), and anhydrous DMF (10 mL) were mixed in a 100 mL round-bottom flask maintained in an ice bath. Afterwards, 75 μL EGVE was added and the mixture was kept at 30 °C for 24 h under nitrogen atmosphere. The crude solid product was purified by dialysis against water using a regenerated cellulose membrane (MWCO: 2000 Da) to obtain mPEG-PLA-EGVE (yield: 63.8%). ^{1}H NMR (400 M, DMSO-d_6), δ[ppm]: δ1.45 (5, m, —CH_3 PLA repeating unit); δ2.50–2.57 (6/7, m, 4H); δ3.24 (1, s, —OCH_3 PEG end group); δ3.51 (2/3, —CH_2 PEG repeating unit,); δ3.84 (9, t, 2H); δ4.00(11, d, 1H); δ4.16 (12, d, 1H); δ4.22(8, t, 2H); δ5.20(4, m, —CH PLA repeating unit); δ6.50 (10, m, 1H) (Fig. 2).

2.4. Synthesis of mPEG-PLA-Ace-Cur

mPEG-PLA-EGVE (1 g, 0.250 mmol), Cur—OH (0.311 g, 0.750 mmol), P-TSA (0.00043 g, 0.0025 mmol), anhydrous DMF (10 mL) were mixed in a 100 mL round-bottom flask with light protection under nitrogen atmosphere (Scheme 1). The reaction was maintained at 50 °C based on a previous report with minor modification [24]. After 4 days, the reaction solution transferred dropwise to ice-cold diethyl ether (200 mL) and the precipitation