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Functional-segregated coumarin-containing telodendrimer nanocarriers for efficient delivery of SN-38 for colon cancer treatment

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ABSTRACT

Four coumarin-containing telodendrimers (denoted as P-I, P-II, P-III and P-IV) were designed and synthesized to self-assemble into the corresponding nanoparticles. Of those, two nanoparticles (P-II and P-IV micelles) were screened and selected for targeted drug delivery of 7-ethyl-10-hydroxy camptothecin (SN-38), a prominent and efficacious anticancer agent, for the treatment of colon cancers. The nanoparticle encapsulation significantly increased the solubility of SN-38 in aqueous solution. Dynamic light scattering (DLS) showed the size of these SN-38 nanoparticles to be around 50 nm, and rod-shaped micelles were observed using transmission electron microscopy (TEM). These two novel nanoformulations of SN-38/P-II and SN-38/P-IV were found to exhibit similar in vitro cytotoxic activity against colon cancer cells as the free drug (SN-38 in DMSO) and were 500-fold more potent than irinotecan (a prodrug of SN-38). In addition, near infrared fluorescent (NIRF) optical imaging was utilized to monitor the tumor targeted delivery of SN-38/NPs via co-loading a NIRF dye. It was demonstrated that these NPs preferentially accumulated in tumors when compared to healthy tissue. A pharmacokinetics study showed that SN-38 micelle formulations had a longer circulating time in blood than irinotecan. Furthermore, SN-38 loaded nanoformulations exhibit superior anti-tumor efficacy when compared with irinotecan at equivalent SN-38 dose in HT-29 human colon cancer xenograft models.

1. Introduction

Cancer remains a cause of considerable morbidity and mortality worldwide, recently surpassing heart disease as the leading cause of death in the US population younger than 85 years old. Colon cancer is a neoplastic disease of the large intestine, one of the most common malignancies in the western world with more than one million new cases each year and a disease-specific mortality rate of about 33% [1]. Although death rates are decreasing in the most developed countries owing to screening programs and therapy, colon cancer still holds third place in cancer death statistics for women and second for men [2]. Chemotherapy is an important treatment option for cancer patients. However the specific delivery of chemotherapeutic drugs to tumors is still a major hurdle in eradicating cancers. The continual development of drug delivery technologies is vital to future breakthroughs in chemotherapy.

Irinotecan (CPT-11), a topoisomerase I inhibitor, has been approved as the first-line chemotherapy drug for colorectal cancer in combination with a 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX) regimen or as a monotherapy for second-line therapy following a failed FOLFOX regimen [3]. 7-Ethyl-10-hydroxy-camptothecin (SN-38) is a biologically active form of irinotecan upon the hydrolysis by carboxylesterases in the liver and in tumors. SN-38 is approximately 100–1000-fold more potent against various cancer cells than CPT-11 [4–8]. Although irinotecan has demonstrated efficacy in practice, it is highly inefficient in delivering active SN-38 to tumor tissue. Studies in humans have shown that only 2–8% of the administered dose of irinotecan is actually converted to SN-38 [9]. In addition, up to 95% of SN-38 is bound to circulating proteins such as albumin, which drastically reduces its bioavailability [10]. Another problem linked to CPT-11 in clinical use is its severe gastrointestinal toxicity and myelosuppression, which constitute its dose limiting toxicity (DLT) [11]. The limitations of irinotecan make SN-38, with its high potency, an attractive molecule for anticancer drug development in colon cancer treatment. However, SN-38 is virtually insoluble in all pharmaceutically acceptable solvents and is unable to be used directly to treat patients [12]. The development of enhanced SN38 delivery systems would allow more efficient and safe treatment.

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Nanoparticles with long circulation times have potential to accumulate in tumor tissue through the often leaky vasculature by the well known enhanced permeability and retention (EPR) effect. More than 40 nanomedicines have been approved for clinical use in the past two decades and among those ~25% were for the delivery of chemotherapeutics [13]. Additionally, more than 40 nanomedicine formulations for chemotherapy are currently in various stages of clinical development [13]. In recent years, a number of nanoparticle drug delivery systems, e.g. amphiphilic macromolecular prodrugs [14,15], liposome formulation [16], dendrimers[17] and polymer micelle formulations[18,19] of SN38 have been investigated for improved delivery to cancer cells and tissues. Although promising progress has been made in SN38 delivery in the literature, the clinical evaluation of these SN38 nanoformulations has been limited, creating a need for further investigation. The ultra-flat aromatic structure of SN38 tends to "sneak out" from nanocarriers to form precipitation or induce large aggregation, which hinders the in vivo application. NK012 is a polymeric prodrug of SN38 based on PEG-poly(glutamic acid) and currently is under clinical trials [20,21], which could assemble into micelles for the targeted drug delivery. However, it still relies on enzymatic or chemical cleavage similar to irinotecan, which may reduce the efficacy in treating cancers.

Compared with dendrimers, liposomes and other organic/inorganic nanoparticles, polymeric micelles are much more versatile to deliver a broad spectrum of therapeutics due to their enormous chemical variation, relative easy preparation, multiple functionality and high drug loading capacity [22–25]. Due to their unique size range (10–100 nm), micelles are able to avoid renal clearance with the minimized liver and spleen uptake. These micelles also preferentially accumulate in solid tumors via the EPR effect [26]. In our previous studies, we have designed and synthesized a unique class of well-defined amphiphilic linear-dendritic block copolymers (named as telodendrimer) by stepwise peptide condensation approach. Telodendrimers are composed of linear poly(ethylene glycol) (PEG), dendritic polylysine and specific peripheral groups, which self-assemble into nanocarriers for efficient drug delivery [27–32]. Herein, we rationally designed and developed a series of novel nanocarriers by introducing bio-sourced aromatic coumarin on the periphery of telodendrimer. The inclusion of coumarin is expected to interact with SN-38 via pi–pi stacking and therefore optimize the drug loading capacity and stability.

2. Materials and methods

2.1. Materials and instruments

SN-38 was purchased from AK Scientific, Inc. (Union City, CA, USA). Mono-methyl terminated poly(ethylene glycol) monoamine (MeO-PEG-NH_2, M.W.: 5000 Da) was purchased from JenKem Technology, USA Inc. (Allen, TX, USA). (Fmoc)Lys(Boc)-OH and (Fmoc)Lys(Fmoc)-OH were purchased from AnaSpec Inc. (San Jose, CA. USA). 1-Hydroxybenzotriazole (HOBt, 98%) and *N*,*N*′-diisopropylcarbodiimide (DIC, 99%) were obtained from Acros Organics. The MALDI matrix α-cyano-4-hydroxycinnamic acid was purchased from Sigma Aldrich Chemical Co. 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate (DiD), a hydrophobic near infrared fluorescence dye and 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI) were purchased from AAT Bioquest, Inc. (Sunnyvale, CA). CellTiter 96® AQ_{ueous} MTS reagent powder was purchased from Promega (Madison, WI, USA). Cholic acid and all other chemical reagents were purchased from Sigma–Aldrich. Dialysis membrane with MWCO of 3500 Da was purchased from Spectrum Laboratories, Inc. A disulfide bond containing linker molecules was synthesized following the procedure reported in a literature [33]. Proton NMR spectra were recorded on a Bruker AVANCE 600 MHz spectrometer. Mass spectra were acquired using a Bruker REFLEX-III MALDI-TOF mass spectrometer, equipped with a nitrogen laser delivering 3 ns laser pulses at 337 nm. SN-38 concentration was measured by a Hitachi F-4500 Fluorescence Spectrophotometer.

The nomenclature of the telodendrimers followed the system used in the previous studies: For example, telodendrimer $PEG^{5K}Co_8$ indicates that the molecular weight of PEG is 5 kDa and there are 8 coumarins conjugated on the periphery of dendritic polylysine, three layer telodendrimer PEG^{5K}-CA_4-LO-LS_4Co_4 indicates that four coumarins are conjugated on the periphery via linker molecules containing S–S bonds (LS), and four cholic acid molecules are conjugated in the middle layer of the dendritic polylysine and a triethylene glycol linker (LO) molecule was inserted between two layers. Among the abbreviations are PEG (poly(ethylene glycol)), CA (cholic acid), Co(coumarin), the disulfide linker (LS) and triethyleneglycol linker (LO).

2.2. Synthesis of coumarin carboxylic acid derivative

The coumarin-based carboxylic acid was synthesized according to the literature [34] with some modification. The detailed synthesis procedure was as follows: 2-bromoactic acid (7.9 g, 56.8 mmol), 7-hydroxy-4-methylcoumarin (2.0, 11.4 mmol), potassium carbonate (15.7 g, 113.8 mmol), a trace of potassium iodide and 200 mL of ethanol were placed into a 500 mL round bottom flask equipped with a magnetic stir and refluxed for 20 h. The mixture was then poured into 200 mL of water, followed by pH adjustment to a value of ~5 by addition of hydrochloric acid (5 wt.%). The ethanol in the mixture was next evaporated at room temperature until a precipitate appeared. The final product was obtained at 85% yield by filtration and washed three times with water. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.7 (d, J = 7.7 Hz, 1 H, Ph-H), 6.97 (dd, J = 8.7, 2.7 Hz, 1 H, Ph-H), 6.93 (d, J = 2.5 Hz, 1 H, Ph-H), 6.22 (s, 1 H, C=CH), 4.79 (s, 3 H, CH3).

2.3. Synthesis of telodendrimers

The telodendrimers containing the coumarin derivative (namely as P-I: $PEG^{5K}Co_8$, P-II: PEG^{5K}-CA_4-LO-Co_4, P-III: PEG^{5K}-CA_4-LO-LO_4Co_4 and P-IV: PEG^{5K}-CA_4-LO-LS_4Co_4 respectively; nomenclature: name the abbreviations: PEG (poly(ethylene glycol), CA (cholic acid), LO, LS, Co(coumarin)) were synthesized using a solution-phase condensation reaction starting from MeO-PEG-NH_2 (5000 Da) via stepwise Fmoc peptide chemistry following the previous procedure [29,31]. The typical synthesis of PEG^{5K}-CA_4-LO-Co_4 was as follows:

(Fmoc)Lys(Boc)-OH was coupled onto the terminal amino group on PEG using DIC (3 equivalent) and HOBt (3 equivalent) as coupling reagents at room temperature until a negative Kaiser test result (yellow) was obtained indicating the completion of the coupling reaction. PEGylated molecules were precipitated by pouring reaction solution into excess amounts of cold ether, followed by centrifugation and then three times of washes with cold ether. The white powder precipitate was dried under reduced pressure and the Fmoc protecting group was removed using 20% 4-methyl piperidine solution in DMF. The second coupling of (Fmoc)Lys(Boc)-OH was performed to react with the free amino group on the polymer. After the removal of Fmoc groups, Fmoc protected triethylene glycol linker molecule was coupled to the terminal amino groups. Then two respective coupling of (Fmoc)Lys(Fmoc)-OH were carried out to subsequently generate a dendritic polylysine terminated with four Fmoc groups and two Boc-protected amino groups at the adjacent sites of the polymer. PEG^{5K}-$(NH\text{-}Boc)_4$-$(NH\text{-}Fmoc)_4$ was obtained via coupling of

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