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Research paper

A new targeted delivery approach by functionalizing drug nanocrystals through polydopamine coating

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ABSTRACT

Tumor target specificity via chemotherapy is widely considered to be very effective on tumor treatment. For an ideal chemotherapeutic agent like Camptothecin (CPT) (CPT is the abbreviation for Camptothecin), improved therapeutic efficacy and high selectivity are equally important. Inspired by adhesive proteins in mussels, here we developed a novel tumor targeting peptide XQ1 grafted CPT nanocrystals with polydopamine coating as a spacer. In this study, CPT nanocrystals were coated by polymerization of dopamine that was induced by plasma-activated water under an acidic environment, and then the tumor targeting peptide was grafted onto polydopamine (PDA) (PDA is the abbreviation for polydopamine) coated CPT nanocrystals through catechol chemistry. The PDA layer had negligible effects on drug crystallinity and structure but resulted in drug nanocrystals with excellent dispersion properties, improved dissolution rate and drug stability by preventing water hydrolysis. More importantly, tumor targeting peptide XQ1 facilitated a rapid cross-membrane translocation of drug nanocrystals via receptor-mediated endocytosis, leading to efficient intracellular drug delivery. Moreover, this novel drug formulation demonstrated more potent anti-cancer activity against tumor cells in comparison with free CPT and naked CPT nanocrystals and exhibited high selectivity, all of which are attributed to the tumor target specificity property and inherent pH-dependent drug release behavior.

1. Introduction

Tumor target specificity via chemotherapy is widely considered to be very effective on tumor treatment. For an ideal chemotherapeutic agent, excellent therapeutic efficacy and high selectivity between tumor and normal cells are equally important. The construction of a tumor-targeting drug delivery system not only increases therapeutic efficiency but also diminishes the unexpected side effects [1]. Two tumor-targeting strategies are usually adopted including passive targeting which takes advantage of the enhanced permeability and retention (EPR) effect of poorly developed tumor vessels, and active targeting delivery like the receptor-mediated endocytosis which involves the over-expression of receptors on the surface of tumor cells [2,3].

There are many receptors over expressed on the surface of tumor cells including transferrin-receptors, human epidermal growth receptors and epidermal growth factor receptors [4,5]. Transferrin-receptors are reported to over-express on tumor cells, about 100-fold more than that on normal cells and binding of special ligand to this receptor will trigger endocytosis [6,7]. Consequently, this type of receptor could be regarded as the target and the ligand-grafted anti-cancer drug delivery system could be designed to enhance drug accumulation in tumor tissues and reduce the toxic effect on normal tissues.

Camptothecin (CPT), a hydrophobic plant alkaloid extracted from *Camptotheca acuminate*, is a potential anticancer agent against a variety of cancers. However, its clinical trials were hampered due to the drug's poor aqueous solubility, instability and unexpected side effects [8,9]. To combat these problems, the most outstanding approach is the preparation of drug nanocrystals, which improves the dissolution rate suggested by the Noyes-Whitney equation [10] and the nano-scaled nature enables it to take good advantage of EPR effect [11]. Previous research revealed that CPT nanocrystals have similar, if not better, anti-cancer effects compared to free solvated CPT. However, because of the lack of target specificity, CPT nanocrystals exhibited lower cellular uptake and showed limited therapeutic efficacy towards specific types of tumors [12].

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To the extent of our knowledge, traditional formulations for tumor targeted delivery of CPT include surface-modified silica nanoparticles (SNP) [13], antibody-drug conjugates [14], PEG grafted liposome based carriers [15] and conjugation of antibodies to tumor-specific epitopes like anti-HER-2 immunoliposomes or anti-Fas micelles. Unfortunately, these delivery systems have a variety of limitations. The drawbacks of using monoclonal antibodies as targeting moieties for conjugates are the high cost of the antibody, the comparatively low reproducibility of the synthesis, the complicated structures that are typically obtained and the risk of immunogenicity. Liposome based or polymer based drug carriers often result in poor control over the release of drug, low encapsulation efficiency and poor stability. By contrast, the targeting peptides do not rely on the antigenicity of the target molecule and can be more flexible in binding to locations on single molecules or complex target binding sites that might be sterically hindered from more bulky antibodies [16]. Moreover, the drug nanocrystals formulations are almost pure solid drug with slight stabilizing surfactant that contributes to almost 100% drug loading. However, only few reports have studied tumor-targeting peptide functionalized drug nanocrystals to efficiently and specially deliver CPT.

In this study, we functionalized CPT nanocrystals through the spontaneous polymerization of mussel inspired molecule dopamine [17,18]. Besides the added benefits attributed to polydopamine (PDA) layer such as the improved dissolution rate and stability of CPT, the PDA layer provided a versatile platform for introducing secondary components for diverse functional applications [19,20]. Here, successful immobilization of a modified T7 peptide, with a spacer (XQ-1, HAIYPRHGGGF), to PDA layer via catechol chemistry occurred, the peptide XQ1 still maintained high affinity to transferrin receptors [2,13]. This newly prepared formulation had dramatically improved stability, dispersion property, dissolution rate and showed targeting capability to transferrin receptor over-expressed cancer cells like A549 and Hela. Compared to other functionalization methods, the use of primer PDA is green, facile and more economic [21,23]. This new formulation method using PDA can be a unique and powerful tool for targeted therapy.

2. Experimental section

2.1. Materials

Dopamine hydrochloride(purity > 99%) and Camptothecin (CPT) powder (purity > 99%) were supplied by Sigma Aldrich Company (St. Louis, MO, USA). DMSO (dimethyl sulfoxide, ACS grade) was purchased from Fisher Scientific Corporation (Pittsburgh, PA). Cells and cell culture related products were purchased from Invitrogen (Carlsbad, CA, USA). Whatman nucleopore polycarbonate track-etched membranes were purchased from Whatman Company (Pittsburgh, PA, USA). Other reagents, if not mentioned, were all purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Peptides XQ1 (HAIYPRHGGGF, >90% in purity) were synthesized by Genescript Corporation (Piscataway, NJ). Peptides were dissolved in 1 × PBS to form 5.0 mM stock solutions.

2.2. Preparation of the primer: Polydopamine (PDA) coated CPT nanocrystals

An anti-solvent precipitation augmented by sonication was used to produce PDA coated CPT nanocrystals or Naked CPT nanocrystals [12]. In a typical experiment, 0.1 mL 10 mg/mL CPT DMSO stock solution was added to 10 mL D. I. water (pH = 4). The mixture was irritated by intense sonication followed by rapid stirring (500 rpm for 10 min, 300 rpm for 30 min, and 100 rpm for 3 h) at room temperature. The CPT nanocrystals were filtered by 50 nm polycarbonate membrane filter and then washed with 5 mL of pH = 4 D. I. water three times prior to being re-suspended in pH = 4 D. I. water (for Naked CPT nanocrystals). For PDA coated CPT nanocrystals, after filtration and purification steps, they were re-suspended by 3 mL plasma-activated water, which, was generated by a non-thermal micro-hollow cathode discharge (MHCD) device under acidic environments (pH < 5.5). To be specific, the MHCD was operated at atmospheric pressure by using air as the working gas. The gas flow and discharge power were controlled at 5 L per min and 100 W, respectively. Discharged plasma was generated by fixing the distance of the discharge probe 5.0 mm above the surface of D. I. water in a 50 mL conical tube for 30 s (data in our lab to be published). Afterwards, dopamine was introduced into the water with final concentration of 0.5 mg/mL. This mixture was left at room temperature for 1, 3, 5, 7, and 24 h, respectively, to allow for dopamine self-polymerization and adhesion before being filtered through 50 nm polycarbonate membranes filter and washed with 5 mL of pH = 4 D. I. water for three times. Moreover, the same procedure was conducted without dopamine introduced, which was used as control group. Final product was re-suspended in known volume of pH = 4 D. I. water for storage. CPT content in both Naked and PDA coated CPT nanocrystals was quantified using fluorescence intensity quantification assay against a calibration curve.

2.3. Physico-chemical characterization

Dopamine hydrochloride powder was dissolved in the plasma-activated water under the same conditions as previously stated, which was served as indicator for PDA formation. The reactivity of dopamine was measured at room temperature using UV–vis spectroscopy by following the formation of quinine with running time, to be specific, to measure the UV absorption intensity at 350 nm (characteristic peak of quinine) of different samples [24]. Moreover, Zeta potentials of PDA coated CPT nanocrystals with different formation time together with Naked CPT nanocrystals were measured using dynamic light scattering (DLS) zetasizer (Zetasizer Nano ZS 90, Malvern Ltd., UK).

Surface morphology of both PDA coated and Naked CPT nanocrystals were studied using an established scanning electron microscopy (SEM) analysis. A small volume (20 μL) of two types of nanosuspension was loaded onto the silicon wafer and then air-dried with nitrogen, respectively. The sample was then sputter coated with a conductive layer of gold palladium (Au/Pd) for 1.0 min, resulting in an approximately 15 nm thick coating. SEM images of PDA coated and Naked CPT nanocrystals were acquired at Auriga Modular Cross Beam workstation (Carl Zeiss, Inc.).

In addition, crystallographic patterns of PDA coated and Naked CPT nanocrystals were examined using an X-ray Diffractometer (XRD) (Rigaku, Tokyo, Japan) operated at a scanning rate 0.03°/min from 2θ angle 5–40°.

2.4. Solubility, dispersion and stability

In a typical experiment, free CPT, Naked and PDA coated CPT nanocrystals suspensions were prepared by transferring them into pH = 4 D. I. water with final CPT concentration at 50 μM. An aliquot of suspension were sampled immediately and left at room temperature for 24 h to examine CPT dispersion in medium.

Although CPT is stable in acidic environment like pH = 4, the lactone ring can quickly undergo an opening at physiological pH to lose bioactivity. An established methodology was used to monitor the conversion of CPT from an active form (lactone) to an inactive (carboxylate) form by factor analysis based on steady-state fluorescence spectra [25]. Data from CPT solutions prepared in