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Redox-responsive supramolecular hydrogel based on 10-hydroxy camptothecin-peptide covalent conjugates with high loading capacity for drug delivery

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ABSTRACT

A redox-responsive supramolecular hydrogel system was developed for delivering 10-hydroxy camptothecin (HCPT). The hydrogel was formed by cleaving disulfide bond. The combination of hydrophobic HCPT with hydrogel was a simple and effective way to improve the solubility of HCPT and the drug loading capacity of delivery system. The transmission electron microscopy (TEM) image revealed the self-assembled hydrogel was long and thin nanofibers with a width of <10 nm. Rheological test verified the hydrogel had fine physical properties. *In vitro* release experiment showed that the accumulative releasing percentages within 72 h of HCPT-peptide hydrogels at 3.0%, 4.0%, 5.0% were 16.8%, 21.3%, and 26.8% respectively, which indicated the HCPT-peptide hydrogels had a significantly sustained-release characteristic. Besides, *in vitro* anticancer assay showed that HCPT-peptide hydrogels possessed a favorable anticancer efficacy. These results indicated that HCPT-peptide hydrogel had great potential for cancer treatment as a novel injectable drug delivery system.

1. Introduction

Over the last few decades, hydrogels have been adopted broadly in biomedical fields of wound healing [1–3], tissue engineering [4,5], and drug delivery systems [6–8] due to their high water content, favorable structural features and biocompatibility [9–11]. Supramolecular hydrogels are formed by hydrogelators, which is capable of responding to the environmental condition changes and exhibiting sol-gel transition [12–14]. With the advantages of self-assembly, biocompatibility, syringeability, biodegradability, molecular recognition and diverse functionality, peptide-based supramolecular hydrogels have enormous potential in drug delivery [15–19]. For example, Altunbas and his coworkers developed self-assembly peptide hydrogels which encapsulated curcumin as injectable drug delivery vehicles [20]. *In vitro* experiments indicated that curcumin hydrogels had excellent therapeutic efficacy as localized drug delivery system. Li group conjugated tripeptide derivatives with olsalazine and then small molecular products self-assembled to supramolecular hydrogels in water [21]. The hydrogels could control release of 5-aminosalicylic acid to achieve anti-inflammatory effect. Ren group developed supramolecular hydrogels based on Schiff bases formation [22]. The addition of gemcitabine to the solution of aldehyde-containing short peptides could lead to hydrogels formations. This system could slowly release gemcitabine and had better inhibition capacities to pancreatic cancer cells than free gemcitabine.

Chemotherapy is one of the common strategies in cancer treatment [23,24]. Among various kinds of anti-cancer drugs, paclitaxel [25], cisplatin-based drugs [26] and 10-hydroxy camptothecin [27] have been well recognized, however their clinical applications are limited by the deficiencies of poor water solubility, low bioavailability and systemic toxicity. As supramolecular hydrogels could improve the solubility of anticancer drug in water by virtue of their high water content, researchers have utilized supramolecular hydrogels as drug vehicles for chemical drug delivery [28–30]. Gao group conjugated paclitaxel with phosphatase substrate (NapFFKYp) to obtain the compound of Taxol-NapFFKYp [31]. After the addition of alkaline phosphatase, the compound was transformed into hydrogelator and then self-assembled into supramolecular hydrogel of taxol. It has demonstrated that the hydrogel could be used for cancer therapy as a long-term drug delivery system. Previously, our group reported a new method for synthesizing supramolecular hydrogel precursor (NapFES). The hydrogel precursor was converted to hydrogelator (NapFE) through hydrolysis reaction,

Abbreviations: HCPT, 10-hydroxy camptothecin; GSH, glutathione; HCPT-SA-FFE-ss-EE, HCPT-succinicacid-phenylalanine-phenylalanine-glutamicacid-ss-glutamicacid-glutamicacid; FFEssEE, phenylalanine-phenylalanine-glutamicacid-ss-glutamicacid-glutamicacid; SPPS, solid phase peptide synthesis.

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which self-assembled into NapFE supramolecular hydrogel [32]. The hydrogel could be served as the carrier to load HCPT. This NapFE hydrogel showed recovery and shear-shinning properties, good drug sustained release effect and inhibition capacities to MCF-7 cells, however the low drug loading efficiency was observed. In order to increase the drug loading capacity, reach better sustained release effect and keep favorable anticancer efficacy, we constructed a new drug delivery system based on the covalent combination between HCPT and peptide.

In this paper, we reported a novel supramolecular hydrogel drug delivery system of HCPT. The supramolecular hydrogels were triggered by disulfide bond reduction reaction. In previous reports, pH change, temperature change and enzymatic reaction were usually used to trigger gelation [33,34], while disulfide bond reduction reaction was rarely used [35]. Disulfide bond could be cleaved by glutathione (GSH), which as antioxidant and free radical scavenger widely exists in human body [36]. Thus the GSH-triggered formation of supramolecular hydrogels may be a biocompatible way for constructing a hydrogel system for drug delivery. We constructed a hydrogel precursor (HCPT-SA-FFEssEE), which could be converted to HCPT-peptide supramolecular hydrogel by cleaving disulfide bond by GSH. HCPT-peptide hydrogel was characterized by scanning electron microscope, transmission electron microscope and rheological test. Then *in vitro* drug release and anticancer assay were conducted. The results demonstrated that the novel hydrogel drug delivery system could sustain release of HCPT with a relatively high cumulative release rate and showed a favorable anticancer effect. Besides the HCPT-peptide hydrogel had high drug loading capacity and fine water solubility. These results demonstrated that HCPT-peptide hydrogel could be as an injectable formulation for treatment of cancer.

2. Materials and methods

2.1. Materials

10-Hydroxy camptothecin (HCPT, ≥98.0%) was purchased from Melonepharma (Dalian, China). succinic anhydride (SA, ≥99.0%) was obtained from Lingfeng (Shanghai, China). N-Fmoc amino acids (99.0%) were bought in GLS (Shanghai, China). 4-Dimethylaminopyridine (DMAP, 99.0%), *N*-hydroxysuccinimide (NHS, 98.0%), *N*, *N*-diisopropylcarbodiimide (DIC, 98.0%), *N*, *N*-diisopropylethylamine (DIPEA, 99.0%), cystamine dihydrochloride (98.0%), 1-hydroxybenzotrizole (HOBT, ≥97.0%), trifluoroacetic acid (TFA,99.0%), 1,4-dioxane (≥99.5%), L-glutathione (GSH, 98.0%) were obtained from Aladdin Industrial Corporation (Shanghai, China). MD-MBA-231 human breast cancer stem cells were obtained from America Type Culture Collection (China's district general agent, Beijing, China). MD-MBA-231 cells were grown in RPMI-1640 media containing 10% fetal bovine serum (FBS) (Hyclone, South America), penicillin and streptomycin. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT, ≥98.0%) was gained from Biosharp (U.S.A.).

2.2. Synthesis and characterization

2.2.1. Preparation of HCPT-SA-NHS

The synthesis of HCPT-SA-NHS is illustrated in Fig. 1. First, 0.160 g of HCPT, 0.128 g (3.2 equiv.) of SA and 0.080 g (1.65 equiv.) of DMAP were dissolved in 8 mL of pyridine with stirring in the dark (to prevent the degradation of HCPT) at 55 °C for 24 h. The dissolvent was removed by using a rotary evaporator. The residuum was washed twice with 0.1 mol/L hydrochloric acid. The orange yellow powder of HCPT-SA was obtained after lyophilization with the yield of 80.8% (Mass spectra is shown in Fig. S1). Then 0.150 g of HCPT-succinic acid (HCPT-SA) and 0.074 g (2 equiv.) of NHS were dissolved in 6 mL of DMF, and then 110 μL (2.2 equiv.) of DIC and 0.002 g (0.05 equiv.) of DMAP were added. Then the solution was reacted in a dark place at 55 °C for 24 h, and then 10 mL of water was added to above liquid. After centrifugation, the obtained precipitate was washed three times with water. The brown and yellow powder of HCPT-SA-NHS was obtained after lyophilization with the yield of 68.0% (Mass spectra is shown in Fig. S2).

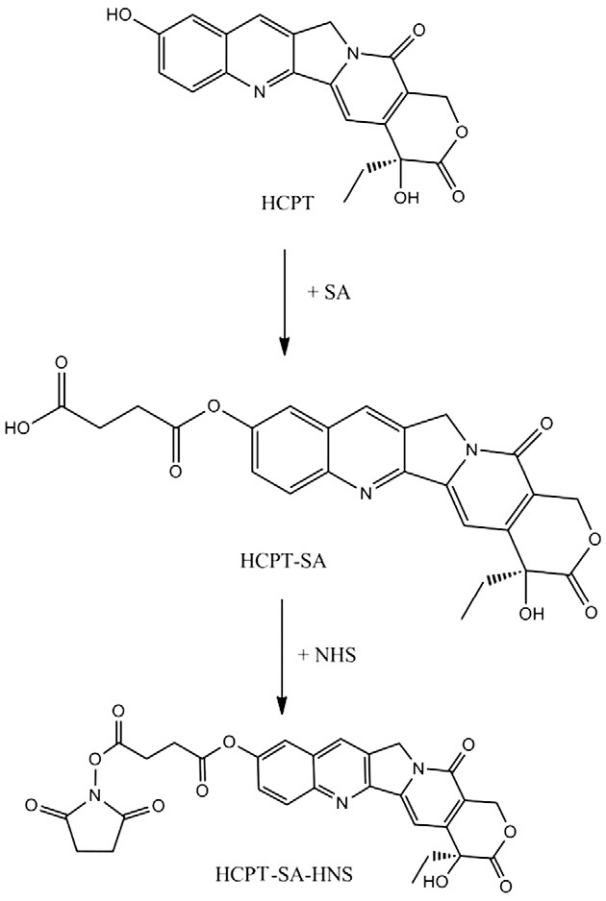

Fig. 1. Synthetic route for HCPT-SA-NHS.

2.2.2. Preparation of FFEssEE

Peptide of phenylalanine-phenylalanine-glutamicacid-ss-glutamicacid-glutamicacid (FFEssEE) was synthesized by a method of solid-phase peptide synthesis (SPPS). First, the N-Fmoc protected amino acid (Fmoc-Glu-OH) was linked to 2-chlorotrityl chloride resin (2-CTA). The resin was washed with DCM three times, followed by DMF five times. DCM/MeOH/DIPEA (80:15:5) was added to cap any remaining reactive chloride group. Anhydrous DMF/piperidine (80:20) was used to deprotect the Fmoc group of the first amino acid. Then TBTU was added to link the second amino acid to the first amino acid. The elongation of peptide FFEssEE chain was followed the standard SPPS method. Finally, 95% trifluoroacetic acid was added to the mixture and then peptide was cleaved from the resin. After filtration, the dissolvent was removed by using a rotary evaporator. Cold ether was added into the residuum and then the sediment was centrifuged for 10 min at 4000 rpm. The white powder of FFEssEE was obtained after lyophilization with the yield of 73.8% (Mass spectra is shown in Fig. S3).

2.2.3. Preparation of HCPT-SA-FFEssEE

67.3 mg of HCPT-SA-NHS and 74.7 mg of FFEssEE were dissolved in 3 mL of DMF, and then 33 μL of DIPEA was added. The solution was reacted in the dark at 55 °C for 24 h. 30 mL of cold ether was added into the mixture solution. The precipitation was centrifuged and treated with water three times and lyophilized. 10-hydroxy camptothecin-succinic acid-phenylalanine-phenylalanine-glutamicacid-ss-glutamicacid-glutamicacid (HCPT-SA-FFEssEE) was obtained by