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COMMUNICATIONS

Small molecular nanomedicines made from camptothecin dimer containing disulfide bond

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Small molecules camptothecin (CPT) dimer could self-assemble into stable nanoparticles in aqueous solution, which was characterized by TEM and DLS. These nanomedicines could be internalized by cancer cells as revealed by confocal laser scanning microscopy, and indicated high cellular proliferation inhibition toward HeLa and HepG2 cells with low IC_{50} values and reduction-responsive cytotoxicity towards HeLa cells. The feasible assembly method and outstanding properties of CPT-NPs provide an alternative approach for exploring new nanomedicines for cancer therapy.

Compared with the most small molecules drugs, nanomedicines offer an opportunity to increase the water solubility, alter the pharmacokinetic profiles of drugs, reduce off-target toxicity, and improve the therapeutic index.[1] Remarkable progress has been made in the development of engineered nanoparticles to treat cancer more effectively. Various nanoparticles have been developed to load antitumor drug and release them in vivo through supramolecular interactions.[2 - 6] For example, the polymeric micelles nanoparticles formed through the hydrophobic interaction of amphiphilic copolymers are one of carriers for drug delivery and release.[7,8] These nanoparticle-based drug delivery systems have indicated improved the antitumor efficacy and reduced the systemic toxicity of chemotherapeutics.[9-11] However, the low drug loading and the potential toxicity of carriers are the major drawback in the translational medicine.[12,13]

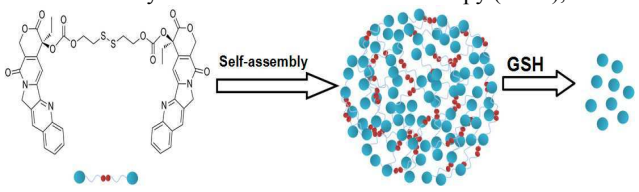
Recently, nanomedicines made directly from small organic molecules draw much more attentions without using of drug carriers. The high drug loading could be easily obtained by choosing hydrophilic short peptide or oligomeric ethylene glycol as the building block.[14-16] More interestingly, Yan et al. reported the amphiphilic drug-drug conjugates and their assembly as nanomedicines for cancer therapy.[17] Very recently, the disulfide-induced self-assembly of hydrophobic molecules provided a new method to make nanomedicine from small organic molecules.[18] As far as we know, no pure drug was used to form the nanomedicine through supramolecular interaction. It is worthy to mention that the CPT dimer was used to enhance the drug loading into polymeric nanoparticles.[19] And Cheng et al. also reported the PEG modified CPT-dimer for the reduction-responsive nanomedicine.[20,21]

In this work, the direct self-assembly of CPT dimer (CPT-s-s-CPT) was demonstrated to form the stable nanoparticles in the absence of surfactants as shown in scheme 1. These nanoparticles possess the glutathione (GSH) responsive drug release and could be internalized by cancer cells and indicated high efficacy of killing HeLa and HepG2 cells.

CPT-s-s-CPT was prepared according to the previous literature.**Error! Bookmark not defined.** Nanoprecipitaiton method was used to prepare CPT-s-s-CPT nanoparticles (CPT-NPs). Briefly, 4 mL of CPT-s-s-CPT (0.36 mg mL^{-1}) solution in N, N-dimethylformamide (DMF) was added into the 10 mL of water at room temperature with vigorous stirring, and then the mixture was dialysed against water to remove DMF. The concentration of the obtained CPT-NPs was determined to be of 34 μg mL^{-1} by UV-vis according to the standard curves (Fig. S1).

Fig.1 shows the UV-vis absorption and photoluminescence spectra of CPT-NPs in water and CPT, CPT-s-s-CPT in DMF solution, respectively. They exhibit similar spectroscopic spectra, but the maximum absorption and emission are slightly different. The maximum absorption peak of CPT-NPs at 355 nm is lower than those of CPT or CPT-s-s-CPT at 364 nm, while the emission peak at 440 nm is larger than those of CPT or CPT-s-s-CPT at 431 nm, indicating the CPT aggregate in particle form.[22] It is not surprise to see almost the same maximum absorption and emission wavelength for CPT and CPT-s-s-CPT. As shown in Fig. S2, CPT-NPs are well-dispersible in water, while CPT and CPT-s-s-CPT don't dissolve in water at the same condition. Under UV irradiation of 365 nm, CPT-NPs emit blue fluorescence.

The morphology and size distribution of CPT-NPs were characterized by transmission electron microscopy (TEM),



Scheme1 The self-assembly and dissociation of CPT dimer.

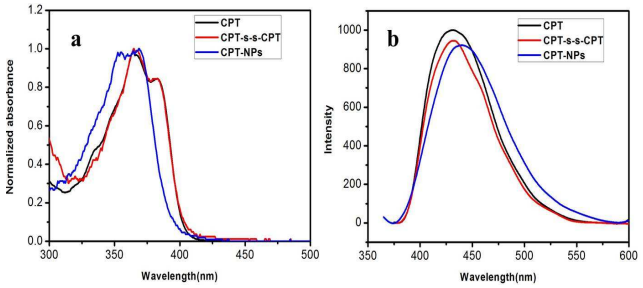


Fig.1 UV-vis absorption (a) and photoluminescence (b) spectra of CPT, CPT-s-s-CPT and CPT-NPs.

scanning electron microscopy (SEM) and dynamic light scattering (DLS). Fig. 2a shows the TEM image of the CPT-NPs. It indicates the isolated spherical particles with an average diameter of 30～50 nm. The SEM image also demonstrates the size of the as-prepared CPT-NPs is approximately 50 nm in diameter (Fig. S3). Average diameter of 53 nm in aqueous solution was confirmed by DLS for CPT-NPs. The size and size distribution do not show obvious changes even in two weeks (Fig. S4). This high stability of CPT-NPs is valuable for their application in nanomedicines.

To demonstrate the disruption of CPT-NPs upon GSH treatment, the change of morphology and size were monitored by TEM and DLS. As show in Fig. 2c, the spherical nanoparticles become irregular, and large agglomerates can be seen after treatment of GSH. The size and count rate of CPT-NPs were monitored over time in Fig. 2d. The size of CPT-NPs increased from 53 nm to 545 nm at 17 h in the presence of 10 mM GSH, due to the break of disulfide bond and aggregation of released CPT. And decreased count rate of CPT-NPs also confirmed the dissociation of nanostructure after GSH treatment. As control, the change of size and count rate was negligible over 17 hours in the absence of GSH, indicating the good stability of CPT-NPs.

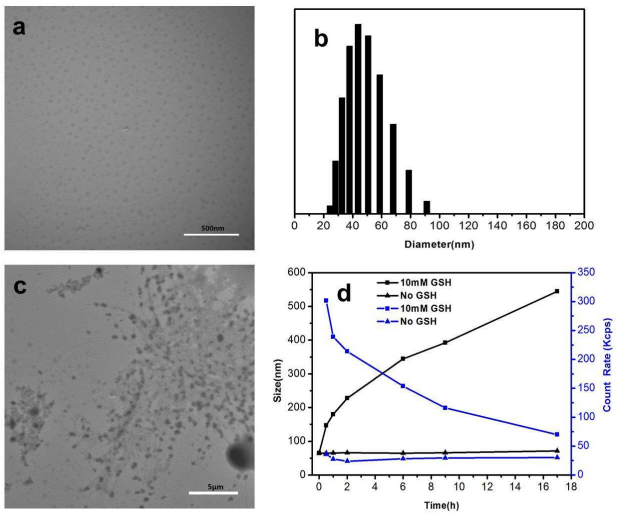


Fig. 2 TEM image (a) and size distribution (b) of **CPT-NPs** determined by DLS, TEM image of CPT-NPs treated with GSH, (d) change of the size and count rate of CPT-NPs over time in the presence of 10 mM GSH.

Cellular uptake of nanomedicines is important for exerting their function. The internalization of CPT-NPs by HeLa cells was evaluated using confocal laser scanning microscopy (CLSM). As shown in Fig. S5, almost no fluorescence was seen after incubation with CPT-NPs for half an hour. Blue fluorescence from CPT can be observed with incubation for 2 h. More interestingly, HeLa cells show the stronger fluorescence upon GSH treatment at the same condition, confirming the GSH responsive drug release in living cells. The cytotoxicity of CPT-NPs was evaluated toward HeLa and HepG2 cells. CPT-NPs indicate effective activity with low IC_{50} of 1.82 μg/ml and 0.44 μg/ml toward HeLa and HepG2 cells, respectively. This high cytotoxicity indicates the formed CPT-NPs still keep the antitumor activity of CPT.

To further confirm the reduction-responsiveness of CPT-NPs for cells, the viability of HeLa cells pretreated with GSH was carried out by MTT. HeLa cells were treated with GSH for 4 h, then were incubated with various concentration of CPT-NPs. As shown in Fig. 4, the cytotoxicity of CPT-NPs was obviously higher in the presence of GSH, compared with that of the untreated groups. The IC_{50} decreased from 1.82 μg/ml to 0.64 μg/ml with the addition of GSH.

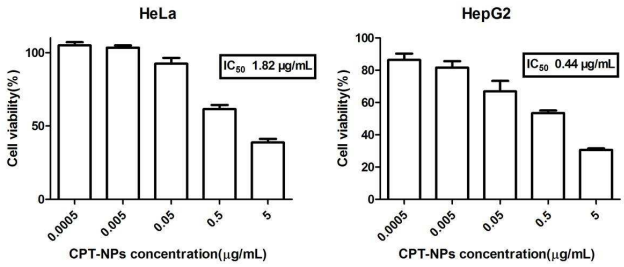


Fig. 3 Cytotoxitity of CPT-NPs toward HeLa and HepG2.

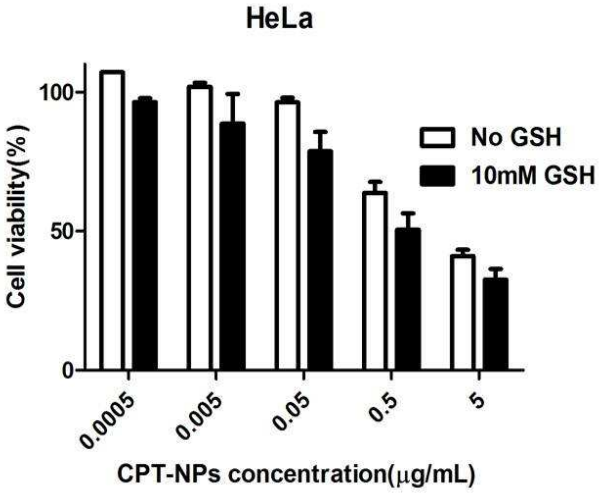


Fig. 4 Reduction-responsive cytotoxicity of CPT-NPs towards HeLa cells.

In summary, CPT nanoparticles were prepared by a precipitation method using a pure organic CPT dimer containing disulfide bond. This disulfide bond-induced self-assembly of organic drug molecules is useful to form nanoscale drug

formulation without loss of antitumor activity. The highly stable and dispersible formulation provides an alternatively new form for traditional chemotherapy drugs. These incredible features make the self-assembly of small molecules drug promising for developing novel nanomedicines in the future.

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Notes and references

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