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The self-assembly of a camptothecin-lysine nanotube

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ABSTRACT

A simple, low molecular weight camptothecin-lysine conjugate is reported to self-assemble into nanotubes with diameters of 70–100 nm and a drug loading level of 60.5%. The nanotubes exhibited promising in vitro cytotoxicity against cancer cell lines A549, NCI-H460 and NCI-H23. The release of active camptothecin was highly dependent on conjugate concentration, temperature and pH of the solution.

The selective delivery of high doses of anticancer drugs to tumor sites without side effects remains as an important goal in cancer therapy.[1] Nanotechnology is emerging as a potential strategy to reduce the dose-limiting toxicity of many chemotherapeutic agents.[2] Linking anticancer drugs to excipient nanoscale carriers[3] exploits the enhanced permeability and retention (EPR) effect to achieve selective transport to tumor tissue.[4] This effect allows nanoscale materials to accumulate in tumor tissues via the abnormally leaky blood vessels at these sites. The majority of nanoscale materials used as inert carriers are polymers,[5] solid nanoparticles,[2c,3b,6] and liposomal aggregates,[7] which bind the drug via noncovalent encapsulation[6a,8] or covalent conjugation.[7a,9] The drug loading level of these constructs is often limited by the size of the inert carrier, which often dominates the mass of the conjugated drug, requiring larger quantities to be administered.[5c,5e] Thus, decreasing the mass of the carrier is necessary to increase the effective drug loading of the nanomedicine. Higher drug loadings have been achieved by the multivalent attachment of the drug to carriers such as dendrimers.[5b,10] An alternative approach creates the nanoscale carrier via the self-assembly of a suitable derivative of the drug.[7b,11] This strategy offers a potential to achieve higher loading levels by using smaller, drug-containing building blocks as precursors.[12]

Camptothecin (CPT) is a natural quinoline alkaloid with potent anti-tumor activity that functions through the inhibition of DNA topoisomerase I.[13] However, the clinical application of CPT is limited due to its poor aqueous solubility, the instability of the E-lactone ring and nonselective biodistribution.[14] Previously, we reported that the assembly of CPT-dipeptide conjugates produced well-defined nanotubes in PBS and human serum.[11c] These nanostructures exhibited enhanced resistance to hydrolytic deactivation and showed high in vitro potency against several human cancer cell types. In this work, we report that a smaller CPT-Lysine conjugate (**A**) assembles into water soluble, uniform nanotubes (Fig. 1) having a high drug loading (≈60.5%) and also exhibits favorable in vitro cytotoxicity and cellular uptake against several tumor cell lines. Furthermore, the potential hydrolytic breakdown products of CPT-lysine **A** would be succinic acid and lysine, which are both classified as 'generally recognized as safe' (GRAS) by the FDA.

CPT-Lysine **A** was prepared via on-resin modification of the ε-amino group of N_{α}-Fmoc-lysine with CPT, linked through a 20-*O*-succinic acid linkage[15] (Scheme S2). In contrast to free CPT, which was insoluble in water (0.003 mg/mL),[16] CPT-Lysine **A** exhibited excellent aqueous solubility (5.14 mg/mL in PBS, pH = 7.4; 12.1 mg/mL in water). The self-assembly of **A** was explored by transmission electron microscopy (TEM) in PBS and pure water. A sample of **A**, incubated at 10 mM in PBS for 72 h, then diluted to 1 mM, exhibited an array of nanotubes displaying diameters ranging from 70 to 100 nm and lengths of several micrometers by TEM imaging (Fig. 2a, S2). When shorter incubation times were employed prior to imaging, a range of intermediates that preceded the formation of well-defined nanotubes could be observed.[17] For example, after 24 h at 10 mM, short, incompletely formed

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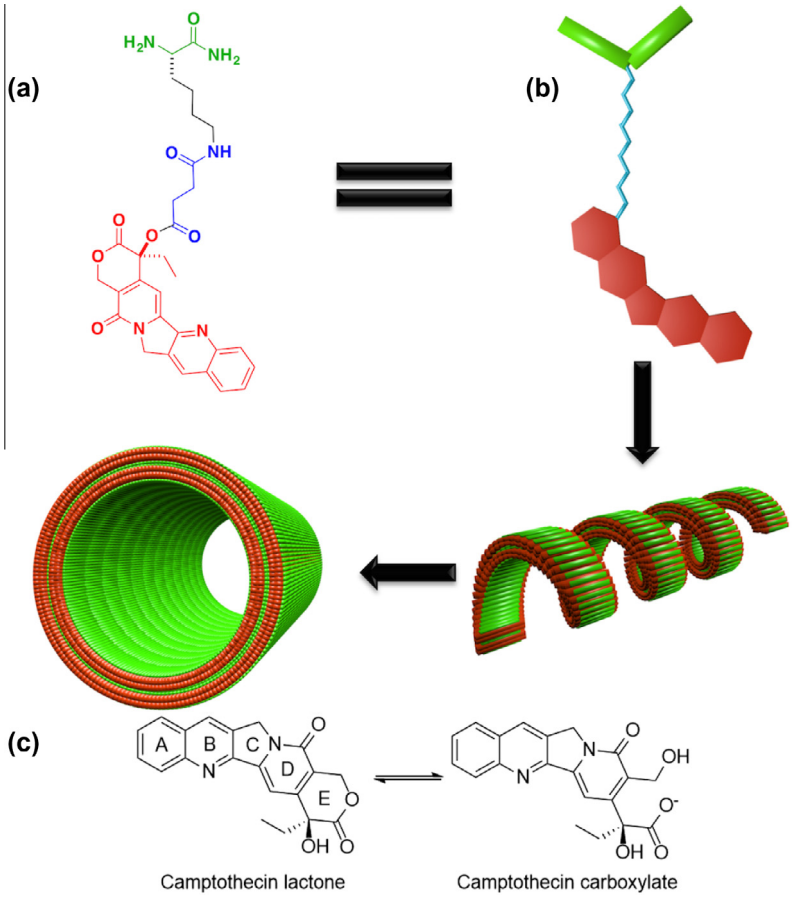

Figure 1. Structure and self-assembly of CPT-Lysine **A**. (a) CPT-lysine **A**. (b) Progressive helical winding and nanotube formation. (c) Hydrolysis of E lactone ring in camptothecin.

nanotubes (<500 nm) were present (Fig. 2b). Under these conditions, partially formed nanotubes, coiled ribbons and helical tapes could be discerned in addition to the fully formed nanotubes. The critical aggregation concentration (CAC) of freshly dissolved solutions of **A** in PBS and H_2O, prepared without preincubation, was 160 and 200 μM respectively, measured using the solvochromatic dye Nile Red (Fig. S4).[18] Solutions of **A** at concentrations in this range (250 μM in PBS), imaged without prior incubation at 10 mM, displayed primarily non-specific aggregation. In pure water (10 mM for 72 h, then diluted to 1 mM, pH 7), **A** formed parallel arrays of nanotubes with decreased diameters (~40 nm) (Fig. 2c). The aligned packing and decreased diameters of the nanotubes can be attributed to the reduced charge screening that takes place in pure water.[19]

The thickness of the nanotube walls (~7.5 nm), as measured by TEM imaging, suggested a double bilayer structure comprised of ~4 molecules of **A** in an extended conformation (1.7 nm) (Fig. 2a (inset), S2). The zeta potential of nanotube **A** in PBS was 19.7 mV, due to the positive ammonium head group of **A**. The UV–Vis spectra of **A** revealed bands at 350 and 368 nm in PBS that were decreased in amplitude and slightly red-shifted compared with solutions measured in TFE, indicative of *J*-type aggregation of the CPT chromophores in PBS (Fig. 2d).[20] Fourier transform infrared spectra of a sample of **A**, prepared in PBS (20 mM), then lyophilized and redissolved in D_2O, exhibited a peak at 1650 cm^{-1}, indicative of the lack of any β-sheet interactions within the assembly (Fig. S1).

The self-assembly of **A** sequesters the hydrophobic CPT structure within the hydrophobic regions of the nanotubes, thereby protecting the 20-*O*-succinyl linkage from the hydrolytic aqueous environment. Free CPT undergoes a reversible, pH-dependent ring-opening of the lactone ring in water, producing the carboxylate form, which has been shown to be toxic.[21] Although hydrolytic cleavage of the 20-*O*-succinyl linkage is required to produce active CPT, competing hydrolysis of the lactone ring of CPT has a detrimental impact on its biological activity.[21c] Accordingly, we explored the capability of the nanotube structure to enhance the stability of the CPT lactone and the 20-*O*-succinyl linkage to hydrolytic cleavage in PBS. The release of CPT from the nanotubes formed from **A** was measured by HPLC over one week as a function of concentration in PBS at 37 °C. Under these conditions, hydrolytic cleavage of 20-*O*-succinyl linkage produced free CPT, observed in both the lactone and carboxylate states.[22] However, the carboxylate form of **A**, which would be formed by CPT-lactone hydrolysis, could not be detected at any concentration or time point. In contrast, exposure of **A** to borate buffer at pH 9 for 6 h, produced free CPT-carboxylate and the carboxylate form of **A**, clearly observable by hplc analysis (Fig. S8). For example, after 3 days at 1 mM in PBS, 61% of CPT was released from **A**, observed in both the CPT-lactone (79%) and CPT-carboxylate (21%) forms. The remainder of **A** (39%) was intact as the lactone without any apparent hydrolysis to the carboxylate form (Fig. S5). Although no apparent ring-opening of **A** could be observed, it is not possible to exclude the possibility that ring-opening takes place prior to a more rapid cleavage of the 20-*O*-succinyl linkage, which would also preclude observation of **A**-carboxylate. The rate of hydrolytic release of CPT from **A** depended strongly on concentration (Fig. 3a). Accordingly, as the concentration was increased from