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Carbon nanotube capsules enhance the *in vivo* efficacy of cisplatin

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ABSTRACT

Over the past few years, numerous nanotechnology-based drug delivery systems have been developed in an effort to maximize therapeutic effectiveness of conventional drug delivery, while limiting undesirable side effects. Among these, carbon nanotubes (CNTs) are of special interest as potential drug delivery agents due to their numerous unique and advantageous physical and chemical properties. Here, we show *in vivo* favorable biodistribution and enhanced therapeutic efficacy of cisplatin (CDDP) encapsulated within ultra-short single-walled carbon nanotube capsules (CDDP@US-tubes) using three different human breast cancer xenograft models. In general, the CDDP@US-tubes demonstrated greater efficacy in suppressing tumor growth than free CDDP in both MCF-7 cell line xenograft and BCM-4272 patient-derived xenograft (PDX) models. The CDDP@US-tubes also demonstrated a prolonged circulation time compared to free CDDP which enhanced permeability and retention (EPR) effects resulting in significantly more CDDP accumulation in tumors, as determined by platinum (Pt) analysis via inductively-coupled plasma mass spectrometry (ICP-MS).

Statement of Significance

Over the past decade, drug-loaded nanocarriers have been widely fabricated and studied to enhance tumor specific delivery. Among the diverse classes of nanomaterials, carbon nanotubes (CNTs), or more specifically ultra-short single-walled carbon nanocapsules (US-tubes), have been shown to be a popular, new platform for the delivery of various medical agents for both imaging and therapeutic purposes. Here, for the first time, we have shown that US-tubes can be utilized as a drug delivery platform *in vivo* to deliver the chemotherapeutic drug, cisplatin (CDDP) as CDDP@US-tubes. The studies have demonstrated the ability of the US-tube platform to promote the delivery of encapsulated CDDP by increasing the accumulation of drug in breast cancer resistance cells, which reveals how CDDP@US-tubes help overcome CDDP resistance.

1. Introduction

Despite some advancement over the past few decades in conventional chemotherapy, the efficacy of the vast majority of cancer therapeutics is often limited for reasons such as low water solubility, rapid elimination, nonspecific biodistribution, rapid breakdown *in vivo*, insufficient accumulation in tumor tissue, undesirable side effects, and development of resistance. These limitations necessitate the development of more efficient ways to administer anticancer drugs systemically to more selectively target tumor tissue, thereby improving efficacy while minimizing undesirable side effects. Over the last decade, with advances in nanotechnology and nanomedicine, numerous nanoparticle-based drug delivery systems have been developed to enhance tumor-specific delivery. One of the major advantages of using nanoparticles as drug delivery vehicle for cancer therapy is that nanoparticles, upon systemic injection, can preferentially accumulate in tumor tissue by taking advantage of irregular tumor vasculature or vessel leakiness. This phenomenon has been termed the enhanced permeability and retention (EPR) effect [1]. Among

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diverse classes of nanomaterials, carbon nanotubes (CNTs) are of special interest in the area of drug delivery due to their numerous unique physical and chemical properties such as high surface area, high aspect ratio, high thermal conductivity, and remarkable optical and electronic properties [2–5]. Owing to their high surface area, CNTs enable the engineering of surface modification for a variety of therapeutic molecules either by specific adsorption or by covalent bioconjugation [6–11]. Due to their hollow cylindrical structure, CNTs also provide internal cavities which are capable of accommodating small molecules or ions [12–14]. While the toxicological effects of CNTs themselves have been widely debated in the literature [15–22], it has been recently shown that appropriately functionalized and highly-purified single-wall CNTs (SWCNTs) can be nontoxic and well-tolerated *in vivo* [23]. Several reports have demonstrated that CNTs readily cross cell membranes due to their intrinsic lipophilic character and high aspect ratio (needle-like structure), and thus are able to transport biological molecules including drug molecules, proteins, plasmid DNA and siRNA into cells [11,24–29].

Over the years, a number of CNT-based drug delivery systems have been explored. In these systems, drug molecules have been mainly attached onto the surface or sidewalls of the nanotubes either by specific adsorption or by covalent attachment [6,11,30,31]. In addition to surface attachment, it has been shown that such drug molecules can be encapsulated within the interior cavity of CNTs [12,13,32–34] to provide an insulating environment for drug molecules. This feature prevents degradation and leakage and other unwanted interaction *in vivo* before the drug reaches its target sites. Recent theoretical studies based on molecular dynamics simulation have also demonstrated that drug molecules can stay within nanotubes for a long period of time during circulation due to the organization of water molecules outside the nanotubes, while the release of drug molecules from nanotubes can be favored near the cell membrane because of advantageous electrostatic interactions of nanotubes with hydrophilic parts of the cell [33,35]. This drug encapsulation approach also preserves the external surface of CNTs for further chemical modification for desired cancer cell targeting with peptide and/or antibodies. Although the ideal length of CNTs for biomedical application is uncertain, ultra-short single-walled carbon nanotubes (US-tubes, $\sim$1.4 nm $\times$ 20–80 nm) which are produced from full-length SWCNTs via a fluorination and pyrolysis procedure (Fig. S1) [36], may be especially well suited for bioactive agent delivery due to their short and relatively uniform-lengths (ca. 95% $\leq$50 nm) which could help them avoid the reticuloendotheial system (RES), while enhancing their cellular uptake properties and eventual elimination profiles. US-tubes, with sidewall defects from the chemical cutting process (Fig. S1) [36], have proven a convenient platform as nanocapsules for the loading and containment of ions, molecules, and drugs whose cytotoxicity may be sequestered within the US-tubes [13,37–40]. Additionally, it has been demonstrated that the exterior surface of US-tubes can be modified with chemical moieties for enhanced solubilization [41], with peptides for biological targeting purposes [42], and with monoclonal antibodies (MAbs) for the specific targeting of cancer cells. Moreover, a medical imaging agent derived from US-tubes (Gadonanotubes) has been shown to translocate into the cells without exhibiting significant toxicity [43]. Finally, one recent study [23] has shown that highly-purified US-tubes are well-tolerated *in vivo* by Swiss mice, even at very high doses ($\sim$0.5 g kg^{-1} b.w.).

We recently developed a new CNT-based drug delivery platform for the treatment of cancer that is comprised of US-tubes loaded with the chemotherapeutic drug cisplatin (CDDP) or (CDDP@US-tubes) [13]. In these previous studies, the encapsulation of CDDP within US-tubes was achieved by a loading procedure that is reproducible as detailed in the Materials & Methods section and as outlined in Fig. S2. The resulting CDDP@US-tube material was characterized extensively by several microscopic and spectroscopic methods (Fig. S3). Moreover, it was also demonstrated that CDDP@US-tubes release CDDP upon dialysis in PBS at 37 °C much more slowly when CDDP@US-tubes are wrapped with Pluronic®-F108 surfactant (W-CDDP@US-tube), which is likely due to the Pluronic wrapping around the US-tubes covering the sidewall defects sites and ends of the US-tubes as a sheath to help prevent premature drug release (Fig. S4). It was shown that the CDDP@US-tube exhibited greater efficacy against MCF-7 and MDA-MB-231 breast cancer cell lines *in vitro*, when compared to free CDDP. Finally, it was also shown that the US-tube platform assists the delivery of encapsulated CDDP into drug-resistant cells, which suggests that CDDP@US-tubes help to overcome CDDP resistance by increasing drug accumulation in otherwise resistant cell. In this study, we report *in vivo* biodistribution and therapeutic efficacy of cisplatin encapsulated within US-tubes (as CDDP@US-tubes) using three different human breast cancer xenograft models.

2. Materials and methods

2.1. Sample preparation

US-tubes, CDDP@US-tubes and pluoronic-F108-wrapped CDDP@US-tubes (W-CDDP@US-tubes) were prepared as previously reported [13]. Briefly, full-length SWCNTs (Carbon Solution Inc.), produced by the electric-arc discharge method, were cut into US-tubes by fluorination followed by pyrolysis at 1000 °C under an inert atmosphere [36]. US-tubes were first purified in concentrated HCl for 1 h by bath-sonication to remove amorphous carbon and metal catalyst impurities (nickel and yttrium), then chemically reduced by a metallic Na°/THF reduction procedure to produce debundled US-tubes [44]. Next, the US-tubes were refluxed for 5 min in 6 N HNO_3 and then repeatedly washed with deionized water. The debundled US-tubes were dispersed in deionized water via bath-sonication for 60 min, and then CDDP was added to the US-tube suspension and vigorously stirred for 24 h, which was then left undisturbed overnight whereupon CDDP-loaded US-tubes (CDDP@US-tubes) flocculated from solution. The CDDP@US-tubes were collected by filtration on a glass filter, washed excessively with deionized water to remove all exterior CDDP from outer surface of the US-tubes, as judged from platinum analysis on the filtrate aliquots by inductive-coupled plasma optical emission spectroscopy (ICP-OES) and then dried at 80 °C. The concentration of CDDP within US-tubes was calculated by quantifying the Pt concentration of CDDP@US-tube samples via ICP-OES. As an average weight percentage, CDDP@US-tubes contained 9.80% wt (±0.88) of CDDP. Extensive characterization of this CDDP@US-tubes material has been also reported previously [13]. W-US-tubes and W-CDDP@US-tubes samples were prepared by suspending either dry US-tubes or CDDP@US-tube samples in a 0.17% (w/v) Pluronic®-F108 surfactant solution via probe sonication for 2 min, followed by centrifugation at 3200 rpm for 10 min (3x) to remove unsuspended CDDP@US-tubes. Pluronic®-F108 is a neutrally-charged, non-cytotoxic surfactant that is commonly used to administer CNT materials for *in vitro* and *in vivo* testing.

2.2. Establishment of xenografts and treatment

All animal experiments were performed under a protocol approved by Baylor College of Medicine Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Experimental Animals. MCF-7 and MDA-MB-231 human breast cell line xenografts were generated by inoculation of 1×10^6 of either MCF-7 or