In vivo Biodistribution and Anticancer Activity of Cisplatin Delivered by Ultra-short Carbon Nanotube Capsules


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ABSTRACT SUMMARY
In our efforts to explore remotely-activated nanocarriers for controlled drug release, we have developed carbon nanocapsules comprised of an ultra-short carbon nanotube shell (US-tubes) loaded with cisplatin (CDDP@US-tubes) and covered with a Pluronic surfactant wrapping to minimize passive release. We have recently demonstrated that non-invasive radiofrequency (RF) electric fields activate the CDDP@US-tubes, producing heat that causes Pluronic disruption, which triggers cisplatin release. Furthermore, release-dependent cytotoxicity was demonstrated in human hepatocellular cancer cells, in vitro. In this work, we have tested the in vivo biodistribution and anticancer effects of these CDDP@US-tubes using mice bearing MCF-7 breast cancer xenograft models. Our results indicate that mice treated with CDDP@US-tubes had significantly more cisplatin in their tumor, spleen, and stomach than compared to the control groups. The anticancer results are also significant in that they demonstrate controlled tumor size and increased survivability in mice treated with the CDDP@US-tubes when compared to free-form cisplatin alone. Using these results as a baseline, future studies will investigate remotely triggered release of cisplatin from CDDP@US-tubes via non-invasive RF electric fields for enhanced anticancer therapies.

INTRODUCTION
Maximizing therapeutic efficacy and minimizing adverse effects remain the fundamental goals of drug delivery in cancer therapy. Over the past decade, drug-loaded nanocarriers have been widely fabricated and studied to enhance tumor specific delivery. In developing remotely-triggered nanocarriers, carbon nanotubes are of particular interest due to their ease of surface functionalization and ability to be loaded with chemotherapies. They also exhibit unique RF properties such as enhancement of local E-fields, absorption and dissipation of RF energy as heat, and alignment parallel to the incident E-field. In previous work, we have developed US-tubes that can be remotely triggered by RF irradiation to release CDDP. Mechanistic insight into the process has revealed thermal disruption of a Pluronic coating by US-tube heating in the RF field without appreciable rise in bulk temperature at an RF dose that is minimally toxic to cancer cells. Furthermore, we have observed release-dependent cytotoxicity in human liver cancer cells. However, the efficacy of this nanocarrier (with and without RF) has yet to be tested in vivo and is the basis of the work herein.

EXPERIMENTAL METHODS
CDDP@US-tubes were synthesized as published previously. Briefly, US-tubes (diameter 1.4 nm, length 20-80 nm) were fabricated using a fluorination and pyrolysis process. The defects created in the US-tube sidewalls allow for easy loading of cytotoxic chemotherapeutics, e.g. CDDP, contrast agents (Gd³⁺, I₂), or radioisotope agents (²¹¹AtCl)⁻. 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 human breast xenografts were generated by inoculation of 1x10⁶ MCF-7 cells suspended in 20 µL Matrigel-PBS into the epithelium-free “cleared” fat pads of SCID/Beige mice. Mice were randomly distributed in groups and were administered intraperitoneal (i.p.) injections when the average tumor volume reached 150 mm³. Injections consisted of either saline, empty Pluronic-wrapped US-tubes (W-US-tubes), CDDP dissolved and diluted in saline solution, Pluronic-wrapped CDDP@US-tubes (W-CDDP@US-tubes), and CDDP@US-tubes dispersed in saline solution via bath-sonication (no pluronic). All CDDP concentrations were normalized to be 2.5 mg/kg mouse body weight (as determined by ICP-MS).

RESULTS AND DISCUSSION
Experimental results are depicted in Figure 1. As shown, a time-related increase in tumor volume was observed in all treatment groups except for the CDDP@US-tube treated groups. The mean fold increase in tumor volume (from day 0 to day 28) of the CDDP@US-tube treated mice was significantly lower compared to CDDP alone. ICP-MS analysis revealed that the CDDP@US-tube treated mice have significantly more Pt in their tumor, spleen and
stomach compared to other treatment groups. ICP-MS analysis of the blood samples revealed that CDDP@US-tubes and W-CDDP@US-tubes exhibited a much longer blood circulation time than CDDP, which was quickly cleared from the blood.

Upon administration, it has been shown that CDDP rapidly binds to albumin and other plasma proteins leading to irreversible deactivation of nearly 90% of the CDDP dose before it accumulates in the tumor by the Enhanced Permeability and Retention (EPR) effect. Because this form of CDDP is inactive, it has no biological effects. We attribute higher CDDP uptake in tumor for CDDP@US-tubes due to their prolonged blood circulation time compared to free CDDP, which facilitates substantial tumor targeting by the EPR effect, and an increase in passive release of active CDDP into the tumor.

CONCLUSION

We have shown that US-tubes can be utilized as a drug delivery platform for release and delivery of CDDP. The results demonstrate that CDDP@US-tubes have greater tumor suppression efficacy than free CDDP in MCF-7 breast xenograft tumor models. We suggest that passive tumor accumulation of CDDP@US-tubes in vivo occurs by the EPR effect due to increased blood circulation times. Future studies will investigate further enhancement of anticancer effects through RF-triggered controlled cisplatin release.

REFERENCES


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