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Convection-enhanced delivery of nanodiamond drug delivery platforms for intracranial tumor treatment

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Abstract

This study examined a novel drug delivery system for treatment of malignant brain gliomas: DOX complexed with nanodiamonds (ND-Dox), and administered via convection-enhanced delivery (CED). Drug retention and toxicity were examined in glioma cell lines, and distribution, retention and toxicity were examined in normal rat parenchyma. Efficacy was assessed in a bioluminescence rodent tumor model. NDs markedly enhanced DOX uptake and retention in glioma cells. ND-Dox delivered via CED extended DOX retention and localized DOX toxicity in normal rodent parenchyma, and was significantly more efficient at killing tumor cells than uncomplexed DOX. Outcomes from this work suggest that CED of ND-Dox is a promising approach for brain tumor treatment.

From the Clinical Editor: In this paper, nanodiamonds were utilized to enhance delivery of DOX in a preclinical glioma model using a convection-enhanced delivery method, demonstrating remarkably enhanced efficacy.

Key words: Nanodiamonds (NDs); Doxorubicin (Dox); Convection-enhanced delivery (CED); Glioma

Abbreviations: NDs, Nanodiamonds; DOX, Doxorubicin; ND-Dox, DOX coupled with NDs; CED, Convection-Enhanced Delivery; BBB, Blood brain barrier; DFTB, Density functional-based tight binding; MD, Molecular dynamics; FITC, Fluorescein isothiocyanate; PBS, Phosphate buffered saline; BLI, Bioluminescent intensity; PI, Propidium iodidle; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; Gd, Gadolinium.

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Glioblastoma is the most common and lethal malignant brain tumor. Even with radical surgical resection, radiation therapy and adjuvant chemotherapy, patients have poor clinical prognosis, with a median survival time of less than 1.5 years.[1,2] To identify new therapeutic strategies this study examines convection enhanced delivery (CED) of DOX complexed with nanodiamonds (ND-Dox), in a rodent brain tumor model. CED is expected to overcome obstacles associated with the treatment of glioblastoma; the lack of penetration of the blood–brain barrier (BBB) and insufficient concentration of the agent in tumor tissue after systemic administration.[3] Conjugation of agents with NDs is expected to increase drug efficacy.

DOX is a cornerstone of chemotherapy regimens, commonly used to treat systemic cancers,[4–7] as well as showing promising therapeutic efficacy in experimental brain tumor models when locally administered.[8,9] DOX has not been considered for clinic chemotherapeutic treatment of malignant brain tumors due to poor penetration across the BBB. Several methods including CED, transiently open the BBB, allowing chemotherapeutic agents access to malignant cells.[10–12] CED can bypass the BBB to deliver therapeutic agents to the brain tumors; reduce systemic drug levels to minimize side effects; and provide prolonged elevation of intracerebral chemotherapeutic agents relative to systemic administration.[13,14] Our laboratory[15,16] and others[17–21] have confirmed that CED is an effective delivery method for brain tumor treatment. However, even with this delivery method, sustained localized distribution and prolonged retention after drug delivery remain major challenges.[22] Localizing drug levels inside the tumor for a prolonged period would increase tumor killing efficacy and improve prognosis. This work examined the hypothesis that these goals are attainable with ND-complexed DOX.

NDs have been proposed as effective drug delivery vehicles.[23–25] These small chemically inert molecules posses uniquely faceted surfaces[26,27] that release drugs in a sustained manner, while improving therapeutic tolerance. Significant efforts have been devoted to understanding the chemical properties of NDs facets and how these properties mediate enhanced drug binding and release. Density functional-based tight binding (DFTB), and molecular dynamics (MD) simulations, among other techniques, have been utilized to study charge distributions on the NDs surface and their role in coordinating the interaction of polymeric and therapeutic compounds.[27–29] Chemical groups at the NDs surface include amine, amide, alcohol, carbonyl, and carboxylic acid groups, with carboxylic acid groups being the most prevalent.[29,30]

NDs exhibit excellent biocompatibility in multiple cell lines and *in vivo,*[31–33] and effectively deliver drugs relative to the spectrum of carbon-based nanomaterials.[34] ND-Dox complexes have been characterized for the treatment of multiple drug resistant tumors.[35] NDs reversibly bind DOX, and allow for sustained functional drug release. Systemic administration of ND-Dox results in virtually no myelosuppression and even lethal doses of unmodified DOX are rendered highly efficient following ND binding. In addition to providing a safe drug delivery system, treatment with ND-Dox significantly reduces tumor size and improves survival time.[35]

Although previous studies have examined the efficacy of CED for brain tumor treatment, this is the first study to: (i) examine CED in combination with ND-drug conjugation and (ii) compare the distribution and toxicity of unmodified vs. ND conjugated drug action in the brain. Conjugation with NDs enhanced DOX uptake and retention in glioma cell lines and in normal rodent parenchyma. Furthermore, when administered via CED, conjugation with NDs localized toxicity and significantly extended DOX brain tumor killing efficacy.

Methods

Chemicals and tumor cell lines culture

See Supplementary Information.

In vitro uptake

To visualize uptake of DOX and ND-Dox, 1×10^4 C6 and U251MG cells were seeded in 8-well chamber slides (BD Company, NJ, USA) and cultured for 24 h. The cells were washed twice with serum-free medium and incubated with fluorescein isothiocyanate (FITC) (5 μg/ml), NDs(5 μg/ml)-FITC, DOX(1 μg/ml) or ND(5 μg/ml)-Dox(1 μg/ml) in serum-free medium for 1 h at 37 °C, then the cells were washed with phosphate buffered saline (PBS) and incubated with culture media. At 4 h, supernatant was removed and the cells were fixed in 4% paraformaldehyde (4 °C, 10 min). Nuclei were stained with DAPI (Invitrogen, NY, USA) (20 °C, 5 min). Cells were visualized with a Zeiss 510 confocal laser scanning microscope using a 63 N (NA1.32) objective. Reflection images were obtained simultaneously with fluorescent images of FITC, ND-FITC, DOX and ND-Dox.

To establish cellular drug uptake profiles, approximately 5×10^3 cells/well were seeded onto 48 well plates, and incubated overnight. Culture media (negative control), DOX(1 μg/ml) or ND(5 μg/ml)–Dox(1 μg/ml) were added to each well. At predicted time intervals (0–4 h), supernatant was removed, cells were washed with ice-cold PBS without iron and lysed with PBS containing 1% triton X-100. DOX concentrations in cell lysates were measured using a Wallac Victor$^{2\,TM}$ 1420 multilabel counter (Perkin Elmer, MA, USA) at an excitation wavelength of 478 nm and an emission wavelength of 594 nm. To adjust for background fluorescence from cellular components, DOX standardization curves were prepared using untreated cells lysed with PBS/1% Triton X-100 containing DOX(1 μg/ml) or ND(5 μg/ml)–Dox(1 μg/ml). DOX and ND-Dox standardization curves were prepared for each experiment. Cellular DOX uptake is expressed as nanomoles per milligram of protein. Protein concentrations of cell lysates were determined with the BCA protein Assay Kit (Thermo Scientific Co., MA, USA).

In vitro retention

To visualize retention of DOX and ND-Dox, C6 and U251MG cells were plated on 8-well chamber slides (BD Company, NJ, USA) (1×10^4/well) and cultured for 24 h at 37°C. The cultures were synchronized for 2 h with serum free medium, after which DOX(2 μg/ml) or ND(10 μg/ml)-