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Intravenous delivery of camptothecin-loaded PLGA nanoparticles for the treatment of intracranial glioma

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ABSTRACT

Effective treatment of glioblastoma multiforme remains a major clinical challenge, due in part to the difficulty of delivering chemotherapeutics across the blood–brain barrier. Systemically administered drugs are often poorly bioavailable in the brain, and drug efficacy within the central nervous system can be limited by peripheral toxicity. Here, we investigate the ability of systemically administered poly (lactic-*co*-glycolic acid) nanoparticles (PLGA NPs) to deliver hydrophobic payloads to intracranial glioma. Hydrophobic payload encapsulated within PLGA NPs accumulated at ~10× higher levels in tumor compared to healthy brain. Tolerability of the chemotherapeutic camptothecin (CPT) was improved by encapsulation, enabling safe administration of up to 20 mg/kg drug when encapsulated within NPs. Immunohistochemistry staining for γ-H2AFX, a marker for double-strand breaks, demonstrated higher levels of drug activity in tumors treated with CPT-loaded NPs compared to free drug. CPT-loaded NPs were effective in slowing the growth of intracranial GL261 tumors in immune competent C57 albino mice, providing a significant survival benefit compared to mice receiving saline, free CPT or low dose CPT NPs (median survival of 36.5 days compared to 28, 32, 33.5 days respectively). In sum, these data demonstrate the feasibility of treating intracranial glioma with systemically administered nanoparticles loaded with the otherwise ineffective chemotherapeutic CPT.

1. Introduction

Malignant gliomas are the most common form of primary brain tumors, afflicting as many as 12,000 patients per year in the United States (Friedman et al., 2000; Grossman and Batara, 2004). Glioblastoma multiforme (GBM) tumors, a grade IV astrocytoma, are distinguished by their fast growing and infiltrative nature. Even after aggressive treatment, which includes tumor resection, radiation, and chemotherapy, the median survival for patients diagnosed with GBM is only 12–14 months (Yang et al., 2014), and few new treatments have advanced to the clinic in the past three decades.

One major challenge to achieving better treatment of GBM is the difficulty of delivering drugs across the blood–brain barrier (BBB), a network of endothelial cells that present both active and passive barriers to the uptake of systemically delivered agents. Chemotherapeutics capable of crossing the BBB are typically poorly soluble and may clear rapidly, and thus high systemic doses are needed to achieve efficacy. This large systemic dose can often have severe toxic effects on peripheral tissue and organs before a treatment benefit is observed.

Thus, many drugs that could be of interest for treating GBM cannot be delivered in doses that are both effective and safe. For example, camptothecin (CPT), a potent DNA damaging chemotherapeutic, is effective at killing cells *in vitro*, but failed in clinical trials due to dose-limiting toxicities and, ultimately, poor efficacy. CPT is rapidly hydrolyzed at physiological pH from its active lactone form to a 10-fold less active, more toxic carboxylate form, which is cleared rapidly once bound to plasma proteins (Mross et al., 2004).

Encapsulation of therapeutics such as CPT in polymeric or liposomal nanoparticles is one strategy that could be used to improve drug action. Drug that has been encapsulated is effectively solubilized and protected from degradation, which prolongs circulation time and increases bioavailability. For example, poly

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(lactic-*co*-glycolic acid) (PLGA) is a biocompatible and biodegradable polymer that can be formed into nanoparticles for encapsulation and sustained release of drug payloads. PLGA nanoparticles are capable of encapsulating a wide range of active agents for sustained release in biological environments, including CPT (Dawidczyk et al., 2014; Dinarvand et al., 2011; Tosi et al., 2013). CPT potency is improved by encapsulation and sustained release when infused directly into tumors (Cirpanli et al., 2010; Sawyer et al., 2011). However, the question of whether CPT-loaded PLGA nanoparticles are capable of treating tumors within the brain when administered intravenously remains unanswered.

The goal of this work was to evaluate the ability of systemically administered CPT-loaded PLGA NPs to treat intracranial GBM in mice. GL261 is a syngeneic mouse glioma cell line that mimics many of the proliferative, invasive, and diffuse characteristics of human GBM (Jacobs et al., 2011; Newcomb and Zagzag, 2009). The use of luciferase expressing GL261 cells (GL261-luc2) allows us to track tumor growth *in vivo* with bioluminescence and, therefore, NP efficacy in immune-competent C57BL/6 albino mice. Nanoparticles were administered to mice bearing orthotopic GL261-luc2 tumors to evaluate specific payload delivery to tumor, peri-tumor, and healthy brain tissue. Efficacy of free CPT versus CPT encapsulated at two doses was determined by tumor growth and survival to test the hypothesis that encapsulation of chemotherapeutic in a nanoparticle could improve systemic therapy of orthotopic GBM.

2. Materials and methods

Camptothecin (CPT), 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR), dichloromethane (DCM), methanol, dimethyl sulfoxide (DMSO), 10% neutral buffered formalin, E-TOXA-Clean and polyvinyl alcohol (PVA) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Ester terminated poly (lactic-co-glycolic acid) (PLGA) (50:50; inherent viscosity = 0.59 dL/g) was obtained from Lactel (Birmingham, AL, USA). All water used in nanoparticle fabrication was endotoxin free (<0.0050 EU/ml) purchased from G-biosciences (St. Louis, MO, USA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), 0.25% trypsin-EDTA and geneticin selective antibiotic (G-418) were purchased from Gibco Invitrogen (Carlsbad, CA, USA). Greiner T25 tissue culture flasks with filter cap and Costar 96 well assay plates (black, flat-bottom, non-treated polystyrene) were purchased from VWR International (Radnor, PA, USA). Beetle luciferin, potassium salt was purchased from Promega (Madison, WI, UAS). GL261-luc2 cells were a generous gift from Dr. Adrienne Scheck (Barrow Neurological Institute, Phoenix, AZ, USA).

2.1. Cell culture

GL261-luc2 expressing cells were maintained at 37 °C and 5% CO_2 on T25 tissue cultures flasks in DMEM supplemented with glucose, L-glutamine, 10% FBS and G-418 antibiotic. Cells were detached with 0.25% trypsin-EDTA and counted using a cellometer mini (Nexcelom Bioscience, Lawrence, MA, USA) to obtain a final concentration of 50,000 cells/2 μl for tumor inductions.

2.2. Nanoparticle fabrication

Nanoparticles were fabricated in endotoxin-free conditions. All glassware and centrifuge tubes were soaked overnight in a 1% w/v E-TOXA-Clean solution and glassware was baked at 250 °C for 30 min. Nanoparticles were produced by single emulsion-solvent evaporation (McCall and Sirianni, 2013) with slight modification. Briefly, 100 mg of PLGA and either 625 μg DiR or 8 mg CPT was dissolved in 1 ml of a 4:1 DCM: methanol mixture. The dissolved PLGA was added dropwise into 2 ml of 5% (w/v) PVA under vortexing and probe sonicated (Fisher Scientific Model 705 Sonic Dismembrator, Waltham, MA, USA) on ice in 3, 10-s bursts at 40% amplitude. The resulting emulsion was added to 50 ml of 0.3% PVA, and this solution was stirred for 3 h to evaporate solvent. Nanoparticles were collected by centrifugation for 20 min at 20,000 RCF and the resulting nanoparticle pellet was washed three times with DI water. The final nanoparticle pellet was resuspended in 1 ml endotoxin free water containing 25 mg Trehalose, frozen, lyophilized for 48 h, and stored at −80 °C. Blank nanoparticles were made by the same method as above without the addition of CPT or DiR.

2.3. Particle characterization

2.3.1. Sizing and morphology

To visualize surface morphology, lyophilized nanoparticles were mounted on double-sided carbon tape and sputter coated with gold for 30 s at 40 mA. Samples were imaged on a SEM-XL30 Environmental FEG at 10 kV. Nanoparticle diameters were measured with ImageJ (v. 1.48, NIH) for a minimum of 200 nanoparticles taken from 5 images. The hydrodynamic diameter and zeta potential of nanoparticles were determined at a concentration of 1 mg/ml in water by dynamic light scattering (DLS) using a Delsa Nano C (Beckman Coulter, Pasadena, CA, USA).

2.3.2. Drug loading

Loading of CPT and DiR were determined by fluorescence. Nanoparticles were dissolved in DMSO to a concentration of 5 mg/ml. The nanoparticle solution (40 μl) and DMSO (10 μl) were pipetted into a black flat bottom 96 well plate and read on a fluorescent plate reader at the appropriate wavelengths (EX/EM 370/428 nm or 750/780 nm, for CPT or DiR respectively). Three samples were read with technical triplicates averaged. Control curves were constructed by dissolving blank nanoparticles as described above and spiking with known amounts of drug or dye.

2.3.3. Controlled release

The method for measuring release of CPT from nanoparticles was adapted from a method described previously (Deng et al., 2014). Nanoparticles (150 μg) with or without CPT were suspended in 2 ml of 1× PBS and incubated at 37 °C on a shaker. At regular intervals (0.5, 2, 4, 6, 24 and 48 h) samples were removed and centrifuged for 10 min at 20,000 RCF. The nanoparticle pellet was discarded and 970 μl of the supernatant was removed and added to 30 μl of quantification fluid (DMSO: 1N HCL: 10% SDS). Control curves were constructed by spiking blank particle samples with known quantities of CPT for fluorescent readout by the method described above. Three samples were measured for each time point.

2.4. In vivo studies

Nanoparticle brain distribution and tumor treatment efficacy were examined *in vivo* in a total of 64 C57BL/6 albino mice (Harlan Laboratories, Indianapolis, IN, USA). All procedures and animal care practices were performed in accordance with the Barrow Neurological Institute's Institutional Animal Care and Use Committee.

2.4.1. Tumor inductions

Tumor induction protocol followed the methods established by (Abdelwahab et al., 2011) with some modifications. Mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and mounted on a small animal stereotaxic instrument (Model 900, Kopf Instruments, Tujunga, CA, USA). Animal temperature was maintained using a