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MR image-guided delivery of cisplatin-loaded brain-penetrating nanoparticles to invasive glioma with focused ultrasound

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ABSTRACT

Systemically administered chemotherapeutic drugs are often ineffective in the treatment of invasive brain tumors due to poor therapeutic index. Within gliomas, despite the presence of heterogeneously leaky microvessels, dense extracellular matrix and high interstitial pressure generate a "blood-tumor barrier" (BTB), which inhibits drug delivery and distribution. Meanwhile, beyond the contrast MRI-enhancing edge of the tumor, invasive cancer cells are protected by the intact blood-brain barrier (BBB). Here, we tested whether brain-penetrating nanoparticles (BPN) that possess dense surface coatings of polyethylene glycol (PEG) and are loaded with cisplatin (CDDP) could be delivered across both the blood-tumor and blood-brain barriers with MR image-guided focused ultrasound (MRgFUS), and whether this treatment could control glioma growth and invasiveness. To this end, we first established that MRgFUS is capable of significantly enhancing the delivery of ~60 nm fluorescent tracer BPN across the blood-tumor barrier in both the 9 L (6-fold improvement) gliosarcoma and invasive F98 (28-fold improvement) glioma models. Importantly, BPN delivery across the intact BBB, just beyond the tumor edge, was also markedly increased in both tumor models. We then showed that a CDDP loaded BPN formulation (CDDP-BPN), composed of a blend of polyaspartic acid (PAA) and heavily PEGylated polyaspartic acid (PAA-PEG), was highly stable, provided extended drug release, and was effective against F98 cells in vitro. These CDDP-BPN were delivered from the systemic circulation into orthotopic F98 gliomas using MRgFUS, where they elicited a significant reduction in tumor invasiveness and growth, as well as improved animal survival. We conclude that this therapy may offer a powerful new approach for the treatment invasive gliomas, particularly for preventing and controlling recurrence.

1. Introduction

Glioblastoma multiforme (GBM) is an aggressive brain tumor that accounts for 67% of all primary brain tumors [1]. Due to the highly invasive nature of the disease, cancer cells often lie beyond the visible tumor boundary, which makes full surgical resection difficult [2]. A significant percentage (90%) of patients develop tumor recurrence at or near the surgical site and many tumors develop drug resistance [3]. Despite advances in drug development, the five year survival rate for GBM is 12% and has remained virtually unchanged over the past decade [1].

cis-Diamminedichloroplatinum (Cisplatin, CDDP) is a powerful chemotherapeutic used as a first-line therapy for several types of cancer, including testicular, ovarian, bladder and lung cancers [1]. It has also been used as an adjuvant in the treatment of pediatric brain tumors [4]. However, adult GBM patients treated with CDDP suffer severe kidney and neurotoxicity, even at sub-therapeutic drug concentrations [5–7]. Methods that reduce off-target toxicity and/or increase local delivery to permit a decrease in systemic dose would greatly increase CDDP's utility in the treatment of brain tumors [5].

While direct injection of CDDP can improve therapeutic outcomes, this requires an invasive procedure and still produces significant neurotoxicity [8]. Biodegradable nanoparticle formulations can shield healthy tissues from the toxic effects of CDDP, especially during systemic administration, while enhancing therapeutic efficacy [1,9]. When combined with a strategy to increase NP concentration at the target site, this

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drug delivery approach provides the ability to achieve sustained therapeutic CDDP levels in the tumor while minimizing both systemic and local off-target effects.

However, drug delivery to the brain presents unique difficulties [10]. Invasive cancer cells exist within otherwise healthy brain tissue, where they are protected from systemically administered drugs by the blood-brain barrier (BBB). The BBB regulates the transport of most molecules to and from the brain and prevents the vast majority of CNS therapeutics from reaching their target [11,12]. The BBB within the tumor may be impaired, but this impairment is often heterogeneous [2,13] and produces an unfavorable pressure gradient for drug penetration [14]. Additionally, brain tumors have a higher cell density and collagen content than normal brain tissue, further limiting drug penetration [15,16]. Together, these factors generate what is now commonly referred to as the "blood-tumor barrier" (BTB). Transcranial MR-guided focused ultrasound (MRgFUS) is currently the only treatment modality capable of achieving safe, non-invasive, reversible BBB and BTB disruption in a targeted manner [17–22].

Nonetheless, once therapeutics are delivered across the BBB and/or BTB using MRgFUS, they must still penetrate brain tissue through the complex brain extracellular space (ECS) in order to provide more uniform drug delivery to the tumor, including invasive cells. To address this challenge, we recently developed "brain penetrating nanoparticles" (BPNs), which are drug- or DNA plasmid-loaded nanoparticles that possess non-adhesive surfaces (a result of exceptionally dense coatings with poly(ethylene glycol (PEG)) which enable particles up to 114 nm [23] and 70 nm [24] in size to rapidly spread within normal brain parenchyma and brain tumors, respectively. Importantly, when considering the systemic administration of BPN, the dense PEG coat also offers long circulation times because rapid clearance by the reticuloendothelial system (RES) is minimized [25].

In this study, we tested the hypothesis that MRgFUS will increase the delivery of drug-loaded BPN across the BBB/BTB in an intracranial rat model of glioma, yielding reduced tumor invasiveness and improved tumor growth control and animal survival. We used both fluorescently labeled PEGylated polystyrene (PS-PEG) BPN and biodegradable cisplatin-loaded BPNs (CDDP-BPN) composed of PAA/PAA-PEG blends to evaluate the efficacy of the therapy. We show that MRgFUS significantly improves BPN delivery and distribution in the tumor and that MRgFUS in combination with CDDP-BPN improves tumor growth control and animal survival. This is the first MRgFUS study demonstrating efficacy with a systemically administered drug-loaded biodegradable polymeric nanoparticle in the treatment of GBM.

2. Material and methods

2.1. PEGylation of polyaspartic acid (PAA) polypeptide

A *co*-polymer of PAA-PEG was synthesized with 27 kDa PAA (200 apsartic acid units, Alamanda Polymers, Huntsville, AL) of which C- and N-terminus are amide and amine, respectively, and 5 kDa methoxy-PEG-amine (mPEG-NH_2, Creative PEGworks, Winston Salem, NC). Briefly, PAA was reacted with mPEG-NH_2 at a 1:10 M ratio with an addition of 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide (EDC, Invitrogen, Carlsbad, CA; equimolar with PEG). The reaction was carried out in 200 mM borate buffer (pH 8.5) for 24 h at room temperature followed by dialysis against deionized water using a 50 kDa MWCO dialysis device (Spectrum Lab, Rancho Dominguez, CA) for 120 h. The solution was lyophilized to obtain a powder of purified PEG-conjugated PAA (PAA-PEG) which was then stored at −20 °C until use. The PAA:PEG ratio was confirmed using nuclear magnetic resonance (NMR) to be ~1:10: 1H NMR (500 MHz, D2O): δ 2.70–2.80 (br, —CHC$\underline{H_2}$COOH) 3.55–3.75 (br, —CH_2CH_2O—), 4.40–4.55 (br, NHC$\underline{H}$C$\underline{H_2}$—) and 3.3–3.4 (s, —$OCH_2CH_2C\underline{H_3}$). A representative NMR spectrum confirming PEGylation of PAA is provided in Fig. S1. Immediately prior to nanoparticle (NP) formulation, the lyophilized polymers were dissolved in ultrapure distilled water.

2.2. Fluorescent labeling of PAA polymer

For fluorescent labeling of polymer, AlexaFluor 647-cadaverine (AF647; Thermo Fisher Scientific) or AlexaFluor 555-cadaverine (AF555; Thermo Fisher Scientific) was conjugated to PAA in 200 mM borate buffer (pH 8.5) for 72 h at room temperature. The solution was dialyzed against deionized water using a 20 kDa MWCO G2 Dialysis device (Spectrum Lab) for 120 h, followed by lyophilization. The AF647- and AF555-labeled PAA (AF647-PAA and AF555-PAA, respectively) were stored at −20 °C until further use.

2.3. Preparation of CDDP loaded nanoparticles

Un-PEGylated CDDP-loaded NP (CDDP-UPN) were formulated using a previously reported method [27] with a slight modification. Briefly, 5 mM cisplatin and 37.03 μM PAA (equivalent to 5 mM aspartic acid residues) were dissolved in RNase-free water and reacted for 72 h. CDDP-BPN were formulated using previously reported method with appropriate modifications to improve CDDP loading [27]. As a first step, CDDP (16.7 mmol) and silver nitrate (31.73 mmol) were reacted together in 5 mL of nuclease-free water at 55 °C for 3 h and at room temperature for additional 21 h. The reaction mixture was centrifuged at 15000 rpm for 10 min to separate silver chloride precipitate formed during the reaction. The supernatant was filtered through a 0.45 μm filter to obtain aquated cisplatin. The concentration of aquated cisplatin was measured using flameless atomic absorption spectrophotometer (AAS; Perkin Elmer, Waltham, MA) and adjusted to 5 mM. PAA-PEG and PAA were dissolved in RNase-free water at a 17:1 mass ratio to obtain a total aspartic acid residue concentration of 5 mM. The pH of the mixture was then adjusted to 6.5–6.8 using 0.1 M NaOH, and reacted with 5 mM aquated cisplatin at room temperature for 72 h. Subsequently, CDDP-UPN or CDDP-BPN were transferred to a centrifugal filtration unit (Amicon Ultra, 100 kDa MWCO; Millipore, Billerica, MA), and centrifuged at 1000 xg for 10 min. Both CDDP-UPN and CDDP-BPN were stored at 4 °C until further use. Fluorescent CDDP-UPN and CDDP-BPNB were formulated with AF555-PAA and a mixture of PAA-PEG and AF647-PAA, respectively.

2.4. Physicochemical characterization of NP

Physicochemical characteristics of NP were determined using a Zetasizer Nano ZS (Malvern Instruments, Southborough, MA). All particles were diluted in 10 mM NaCl (diluted from phosphate buffered saline) and dynamic light scattering (DLS) was employed to determine the hydrodynamic diameter and polydispersity index (PDI) at a backscattering angle of 173°. The ζ-potential, a measure of particle surface charge, was determined using laser Doppler anemometry. Quantification of drug content within the NP was conducted using AAS and the loading density was calculated as the % mass of drug in the total particle mass. The size and morphology of NP was determined using a Hitachi H7600 transmission electron microscope (TEM, Hitachi, Japan).

2.5. NP stability analysis

NP stability was measured in artificial cerebrospinal fluid (ACSF) and 10% fetal bovine serum (FBS). Either CDDP-UPN or CDDP-BPN were dissolved in ACSF and/or 10% FBS at 37 °C and hydrodynamic diameters were determined by DLS at different time points after the initiation of incubation.