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Convection enhanced delivery of cisplatin-loaded brain penetrating nanoparticles cures malignant glioma in rats

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ABSTRACT

Glioblastoma multiforme (GBM) is highly invasive and uniformly fatal, with median survival < 20 months after diagnosis even with the most aggressive treatment that includes surgery, radiation, and systemic chemotherapy. Cisplatin is a particularly potent chemotherapeutic agent, but its use to treat GBM is limited by severe systemic toxicity and inefficient penetration of brain tumor tissue even when it is placed directly in the brain within standard delivery systems. We describe the development of cisplatin-loaded nanoparticles that are small enough (70 nm in diameter) to move within the porous extracellular matrix between cells and that possess a dense polyethylene glycol (PEG) corona that prevents them from being trapped by adhesion as they move through the brain tumor parenchyma. As a result, these "brain penetrating nanoparticles" penetrate much deeper into brain tumor tissue compared to nanoparticles without a dense PEG corona following local administration by either manual injection or convection enhanced delivery. The nanoparticles also provide controlled release of cisplatin in effective concentrations to kill the tumor cells that they reach without causing toxicity-related deaths that were observed when cisplatin was infused into the brain without a delivery system. Median survival time of rats bearing orthotopic glioma was significantly enhanced when cisplatin was delivered in brain penetrating nanoparticles (median survival not reached; 80% long-term survivors) compared to cisplatin in conventional unPEGylated particles (median survival = 40 days), cisplatin alone (median survival = 12 days) or saline-treated controls (median survival = 28 days).

1. Introduction

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor with high rates of recurrence and a median survival time of <20 months even following aggressive surgical resection and subsequent irradiation and chemotherapy [1]. Temozolomide (TMZ) is the frontline chemotherapy since it is able to cross the blood brain barrier (BBB) and it provides some improvement in patient survival [1,2]. However, TMZ effectiveness is limited by its low potency as an alkylating agent, dose-limiting systemic toxicity due to myelosuppression and lymphopenia [3–5], rapid degradation in the body, and development of drug resistance [2,4,6]. As a result, new chemotherapeutic approaches for GBM are desperately needed [7].

cis-Diamminedichloroplatinum (Cisplatin, CDDP) is a highly potent agent used to treat numerous types of cancer, including testicular, ovarian, bladder and lung cancers [8]. Additionally, systemic CDDP has been widely adopted as an adjuvant therapy for several brain tumors in pediatric patients, such as neuroblastoma and medulloblastoma [9]. However, CDDP administered systemically to adults with GBM causes severe nephrotoxicity and neurotoxicity at sub-therapeutic drug levels [10–13]. Thus, clinical trials for GBM have investigated alternative platinum-based agents (i.e. carboplatin) that are less toxic, but also less effective [14].

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FDA approval in 1996 of Gliadel, composed of bis-chloroethylnitrosourea (BCNU) impregnated into a biodegradable polyanhydride wafer roughly the size of a quarter, proved that local delivery of chemotherapy in the brain can be both safe and effective in the treatment of GBM [15]. Importantly, local delivery eliminates the severe systemic toxicity observed with most systemically-administered chemotherapeutic drugs, including BCNU. However, the effectiveness of Gliadel is limited primarily because BCNU released from the wafers only penetrates a few millimeters into brain tissue [16], but also because BCNU is not a particularly potent chemotherapeutic agent. Intracerebral infusion of CDDP at sub-therapeutic doses was safely conducted in humans with malignant glioma [17,18], but higher doses that might be effective were not tested due to safety concerns. Recent preclinical studies have revealed promising therapeutic outcomes following intratumoral administration of CDDP [19,20], but neurotoxicity of CDDP administered without a drug delivery system remains a significant limitation [20,21]. The encapsulation of chemotherapeutics into nanoparticles (NP) that slowly mete the drug out at therapeutic levels can greatly improve drug safety and efficacy [8,15]. Thus, it is logical to administer chemo-loaded NP locally to treat brain tumors. However, most NP do not penetrate tumors well since they are either too large to fit through the spaces between cells or their surfaces are adhesive to the extracellular matrix (ECM) [22]. Convection enhanced delivery (CED) has been widely explored to facilitate drug distribution in the brain [23,24]. However, most NP still remain at the infusion site even with pressure-driven flow provided by CED [25,26], likely due to the nanoporous and highly adhesive ECM [27,28]. We have previously demonstrated that a dense polyethylene glycol (PEG) corona on NP eliminates adhesive trapping in the brain parenchyma [29], and that such particles rapidly penetrate healthy brain tissue if they are <114 nm in diameter [29] and brain tumor tissue if they are <70 nm in diameter. We termed such particles "brain penetrating particles", or BPN, whereas we call the same particles without a dense PEG corona "un-PEGylated particles", or UPN [29]. Here, we describe the development of CDDP-loaded polymer nanoparticles, with and without a dense PEG corona, that release CDDP in a controlled manner. We then compare the ability of the BPN and UPN to penetrate healthy brain tissue and brain tumor tissue, both ex vivo and in vivo, and to treat rats with orthotopic GBM.

2. Materials and methods

2.1. PEGylation of polypeptide

Poly(aspartic acid) (PAA) with a molecular weight of 27 kDa (Alamanda Polymers, Huntsville, AL) was reacted with PEG with a molecular weight of 5 kDa (Creative PEGworks, Winston Salem, NC) at a 1:10 M ratio, as facilitated by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Invitrogen, Carlsbad, CA) at stoichiometric concentrations, in 50 mM 2-(*N*-morpholino)ethanesulfonic acid (MES Buffer, pH 5.0, Sigma Aldrich, St. Louis, MO). The reaction was carried out for 72 h at room temperature followed by dialysis against deionized water using a 20 kDa MWCO cassette (Spectrum Lab, Rancho Dominguez, CA) for 120 h. The solution was then lyophilized to obtain the PAA-PEG polypeptide which was then stored at -20 °C until use. The PAA:PEG ratio was confirmed through nuclear magnetic resonance (NMR) to be ~1:10 (Fig. S1): ^{1}H NMR (500 MHz, D_2O): δ 2.70–2.80 (br, $CHCH_2COOH$) 3.55–3.75 (br, CH_2CH_2O), 4.40–4.55 (br, $NHCHCH_2$). Immediately prior to NP formulation, the lyophilized polymers were dissolved in ultrapure distilled water.

2.2. Fluorescent labeling

Fluorescent labeling dyes, Alexa Fluor® 555 and 647 (AF555 and AF647, Molecular Probes, Eugene, OR), were conjugated to PAA and PAA-PEG polypeptide, respectively, by dissolving in 200 mM borate buffer (pH 8.2) and reacting for 72 h at room temperature. The solution was dialyzed against deionized water using a 20 kDa MWCO cassette (Spectrum Lab) for 120 h, followed by lyophilization. The labeled polypeptides were stored at −20 °C until use.

2.3. NP preparation and characterization

CDDP-loaded BPN (CDDP-BPN) and UPN (CDDP-UPN) were formulated using the following protocol. The CDDP-UPN were formulated by mixing 5 mM of CDDP (Sigma Aldrich) with 7.5 mM of aspartic acid (i.e. PAA only) in RNase-free water for 72 h at room temperature. For the CDDP-BPN, 5 mM of CDDP was mixed with 5 mM of aspartic acid (i.e. 9:1 aspartic acid ratio of PAA-PEG:PAA) in RNase-free water for 72 h at room temperature. NP were then collected using ultracentrifuge filters (Amicon Ultra, 100 kDa MWCO; Millipore, Billerica, MA) by centrifuging at 1000 xg for 10 min and stored at room temperature until further use. Physicochemical characteristics of NP were determined using a Zetasizer NanoZS (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 surface charge (ζ-potential) of the particles was determined using laser Doppler anemometry. Quantification of CDDP within the NP was conducted through flameless atomic absorbance spectroscopy (AAS) (Perkin Elmer, Waltham, MA) and loading was calculated as the % mass of CDDP in the total sample. NP imaging was conducted using a Hitachi H7600 transmission electron microscope (TEM, Hitachi, Japan).

2.4. CDDP release kinetics

To determine the CDDP release over time, CDDP-UPN or CDDP-BPN were dispersed in 1 mL of artificial cerebrospinal fluid (ACSF) (Harvard Apparatus, Holliston, MA) within a 100 kDa MWCO dialysis membrane (Spectrum Laboratories, Rancho Dominguez, CA). The chamber was then placed in a 14 mL ACSF sink and shaken at 37 °C. At specific time points, the entire sink volume was removed and replaced with 14 mL of fresh ACSF. Samples were quantified using AAS.

2.5. In vitro cell viability

Rat brain tumor cells, including 9 L gliosarcoma and F98 glioma lines, were cultured and passaged in Dulbecco's Modified Eagles Medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin streptomycin (Invitrogen). Cells were seeded at a concentration of 2000 cells/well in 100 μL of media and allowed to attach overnight in 96 well plates. On the following day, the media was replaced with 100 μL of fresh media and 10 μL of either CDDP or CDDP-BPN (20 μM to 0.002 μM in 10-fold dilutions) was administered. Cells were incubated for 3 days at 37 °C and 5% CO_2. To quantify the number of live cells, media was replaced with 100 μL of fresh medium and 10 μL of Dojindo Cell Counting Kit-8 (Dojindo Molecular Technologies, Inc., Rockville, MD) solution was added. Cells were incubated for 2 h at 37 °C, followed by the measurement of absorbance at 450 nm using a Synergy Mx Multi-Mode Microplate Reader (Biotek, Instruments Inc. Winooski, VT). The % cell viability was normalized to the untreated cell control.

2.6. Neocortical slice preparation and multiple particle tracking

Healthy or tumor-bearing rat brain tissue slices were prepared according to a slightly modified protocol of a previously publication [29]. F98 tumor-bearing Fischer 344 rats were sacrificed 12 days after the tumor inoculation (1×10^5 cells) to ensure a significantly large tumor bulk for investigation. Healthy or tumor-bearing brain tissue was sliced into 1.5 mm thick slices using a Zivic Mouse Brain slicer (Zivic