International Journal of Pharmaceutics 517 (2017) 168–174

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Albumin nanoparticles for glutathione-responsive release of cisplatin: New opportunities for medulloblastoma

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ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form 6 December 2016
Accepted 8 December 2016
Available online 9 December 2016

Keywords:
Albumin nanoparticles
Redox responsivity
Glutathione
Biocompatibility
Medulloblastoma
Cisplatin

ABSTRACT

Redox-responsive nanoparticles were synthesized by desolvation of bovine serum albumin followed by disulfide-bond crosslinking with *N*, *N*′-Bis (acryloyl) cystamine. Dynamic light scattering and transmission electron microscopy studies revealed spherical nanoparticles (mean diameter: 83 nm, polydispersity index: 0.3) that were glutathione-responsive. Confocal microscopy revealed rapid, efficient internalization of the nanoparticles by Daoy medulloblastoma cells and healthy controls (HaCaT keratinocytes). Cisplatin-loaded nanoparticles with drug:carrier ratios of 5%, 10%, and 20% were tested in both cell lines. The formulation with the highest drug:carrier ratio reduced Daoy and HaCaT cell viability with IC_{50} values of 6.19 and 11.17 $\mu g\,mL^{-1}$, respectively. The differential cytotoxicity reflects the cancer cells' higher glutathione content, which triggers more extensive disruption of the disulfide bond-mediated intra-particle cross-links, decreasing particle stability and increasing their cisplatin release. These findings support continuing efforts to improve the safety and efficacy of antineoplastic drug therapy for pediatric brain tumors using selective nanoparticle-based drug delivery systems.

1. Introduction

Medulloblastomas (MBs) are among the primary brain tumors most frequently diagnosed in pediatric patients. They occur mainly in infants and children but occasionally also adolescents (or even adults). MBs arise in the posterior fossa and fourth ventricle and have a high propensity to metastasize (Coluccia et al., 2016; Morfouace et al., 2014). Most MBs are still treated with the combined use of aggressive surgical resection followed by multi-agent chemotherapy and, for children over 3 years of age, craniospinal irradiation. Although this approach has considerably improved the 5-year survival rates of patients with MBs, the treatments themselves frequently cause acute and/or long-term adverse effects, many of which severely diminish the survivors' quality of life (Matheson et al., 2016; Nageswara Rao et al., 2014).

For the past 20 years (Parhizkar et al., 2016), the alkylating agent cisplatin (CPT) has been a mainstay of MB chemotherapy. However, its high toxicity often results in major adverse effects, including stunted growth, irreversible hearing loss, and renal dysfunction. These features severely limit the CPT doses that can be used and consequently the therapeutic benefits that can be achieved in children with MB (Dhar et al., 2008; Nageswara Rao et al., 2014; Wehe et al., 2014).

Increasingly dynamic collaboration between oncologists, biologists, and material scientists has generated a number of proposals for improving the safety and efficacy of brain tumor chemotherapy regimens (Gharpure et al., 2015; Kim et al., 2010; Pacardo et al., 2015; Stylianopoulos and Jain, 2015; Wang et al., 2012). One of the most versatile options involves the use of nanoscale polymeric carriers (Eetezadi et al., 2015; Fernandes et al., 2015; Johnstone et al., 2016): they offer a high surface area for drug binding and controllable physical-chemical and biologic properties, which can

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http://dx.doi.org/10.1016/j.ijpharm.2016.12.017
0378-5173/

be exploited to regulate delivery rates of the drug, thereby improving its pharmacokinetics, therapeutic index, and safety profile (Kumari et al., 2016). Several nanoparticles have also been shown to cross the BBB and accumulate in the CNS (Calvo et al., 2001; Ulbrich et al., 2009), essential requisites for carriers destined for use in the treatment of brain tumors.

Human and bovine albumin have both been widely used to create nanoparticle systems for delivering drugs to a variety of organs. The advantages of albumin include a high drug-binding capacity (Elzoghby et al., 2012), biodegradable and biocompatible properties, and the fact that it already has U.S. Food and Drug Administration approval for pharmaceutical applications (Raval et al., 2015). The hyperpermeability of intratumoral blood vessels enhances the delivery of albumin nanoparticles (A-NP) to neoplastic tissues, where they are readily taken up by cancer cells to meet their increased need for amino acids and energy (Neumann et al., 2010). A-NP accumulation in these cells is also promoted by the reduced venous clearance of most solid tumors (Noguchi et al., 1998). Importantly, several unmodified A-NPs have been shown to cross the BBB via adsorptive transcytosis triggered by electrostatic interactions between the polycationic proteins and the negatively charged brain membrane (Hervé et al., 2008), and their use is not associated with any impairment of locomotor, explorative, or cognitive functions (Bergonzi et al., 2016).

Carriers capable of responding to specific conditions within a cell, such as changes in the redox state, pH, or enzyme activity profile, can enhance a drug's efficacy and specificity while significantly reducing its side effects (Qiu and Park, 2012). Solid tumors, for example, frequently present elevated levels of the master antioxidant, glutathione (GSH) (Ilangovan et al., 2002), which can be exploited to trigger targeted release of anticancer drugs. GSH-responsive delivery can be achieved by using *N*, *N*′-bis (acryloyl)cystamine (BAC) as a disulfide-bond-containing cross-linker in the formation of NPs from naturally occurring polymers (e.g. chitosan and chitosan derivatives). In the presence of high GSH levels, the disulfide bridges within the polymer network undergo reduction, a reaction which destabilizes the NP and increases the release of the drug they are carrying (Curcio et al., 2015b).

In literature, several examples of redox-responsive materials based on serum albumin are presented. Wang et al. prepared a self-cross-link strategy to fabricate HSA nanoparticles stabilized by intermolecular disulfide bonds and dissolvable in reducing media (Wang et al., 2013a). In a work of 2015 (Shi et al., 2015), pH- and redox-sensitive nanoparticles were prepared by covalent linkage of a Pt^{IV} prodrug to human serum albumin (HSA) to form a disulfide containing Pt-HSA complex, followed by conjugation to calcium phosphate (CaP) nanoparticles. Upon cellular uptake, CaP is decomposed with pH < 6.0, releasing Pt-HAS, while the Pt^{IV} prodrug is dissociated from HSA in the form of cisplatin after disulfide reduction by GSH. Adriamycin was also linked to HSA using a disulfide-containing spacer to obtain an amphiphilic specimen able to form micellar-like nanoparticles in which the drug release was controlled by GSH concentration (Chen et al., 2015).

Finally, Molina et al. employed a nanoprecipitation method to obtain HSA nanoparticles decorated with the photosensitizer chlorin e6. The nanoparticles were stabilized using disulfide-containing cross-linker to create a smart drug delivery system that is activated only upon nanoparticles degradation in the reducing intracellular environment (Molina et al., 2016).

In this study, we developed and tested redox-responsive bovine serum albumin (BSA)-nanoparticles (RNPs) for use in the delivery of CPT to MB cells. The RNPs were synthesized by means of a de-solvation technique in which BAC was used as GSH-responsive cross-linker. We used transmission electron microscopy (TEM) for morphological characterization of the RNPs and dynamic light scattering (DLS) to assess their size distribution and redox responsivity. To assess the applicability of these RNPs for the treatment of MB, we measured their *in vitro* cytotoxicity in healthy human keratinocyte (HaCaT) and human MB (Daoy) cell lines. Finally, confocal microscopy was used to confirm the effective uptake of the RNPs by both cell lines.

2. Materials and methods

2.1. Materials

The following reagents were purchased from Sigma Aldrich (St Louis, MO): bovine serum albumin (BSA), cystamine dihydrochloride, acryloyl chloride, fluorescein-5-isothiocyanate (FITC), cisplatin (CPT), glutathione (GSH), NaOH, NaCl, reagent-grade ethanol, reagent-grade dimethyl sulfoxide (DMSO), paraformaldehyde (PFA), Triton X-100, Dulbecco's Modified Eagle Medium (DMEM), phosphate-buffered saline (PBS), sodium carbonate buffer, 200 mM glutamine (Gln), 100 mM sodium pyruvate, 100× non-essential amino acids (NEAA), and antibiotic solution (100 units mL^{-1} penicillin and 10000 μg mL^{-1} streptomycin) were from Sigma Aldrich (St Louis, MO). Minimum Essential Media (MEM) and fetal bovine serum (FBS) were from Gibco-Invitrogen (Carlsbad, CA). Phalloidin was from Thermo Scientific (Waltham, MA, USA). The CellTiter 96 AQ_{ueous} One Solution Cell Proliferation assay was from Promega (Madison, WI, USA).

2.2. Cell culture

Human keratinocytes (HaCaT) and human MB (Daoy) cells purchased from ATCC were cultured at 37 °C in a humidified 5% CO_2 incubator. Briefly, HaCaT cells were grown in DMEM supplemented with 10% FBS, 2 mM Gln, and 100 units mL^{-1} antibiotic solution. Daoy cells were maintained in MEM supplemented with 10% FBS, 1 mM sodium pyruvate, 1× NEAA, and 100 units mL^{-1} of antibiotic solution.

2.3. Synthesis of N, N′-Bis (acryloyl) cystamine (BAC)

BAC was prepared as described elsewhere (Sun et al., 2010). Briefly, 50 mL of an aqueous solution (1.0 M) of cystamine dihydrochloride was placed in a 250 mL three-neck flask equipped with a thermometer and two 50-mL dropping funnels. Then, 10 mL acryloyl chloride solution in dichloromethane (15.0 M) and 20 mL NaOH aqueous solution (10.0 M) were simultaneously added, dropwise, at 0–5 °C and allowed to react at room temperature (RT) for 16 h. The BAC thus obtained was purified by recrystallization from ethyl acetate. The reaction yield was 73% ca.

2.4. Synthesis of FITC-BSA

BSA was labeled with FITC, as previously described (Ke et al., 2015). BSA (60 mg) was dissolved in 3.0 mL sodium carbonate buffer (100 mM, pH 9.2), and 0.6 mL FITC solution in sodium carbonate buffer (1.0 mg mL^{-1}) was then added and allowed to react at RT for 3 h. The FITC-BSA thus obtained was purified by dialysis against 5.0 mM phosphate buffer (pH 3.0) for two days.

2.5. Preparation of RNPs

RNPs were prepared using a previously described de-solvation method with minor modifications (Wolak and Thorne, 2013). BSA powder (200 mg) was added to 200 mL distilled water. The pH was then adjusted to 10 with 0.1 M NaOH while the mixture was stirred at 500 rpm, and the NaCl concentration was fixed at 10 mM. The