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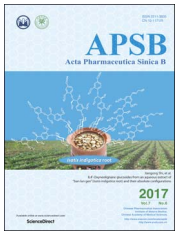

ORIGINAL ARTICLE

Substance P-modified human serum albumin nanoparticles loaded with paclitaxel for targeted therapy of glioma

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Abstract The blood–brain barrier (BBB) and the poor ability of many drugs to cross that barrier greatly limits the efficacy of chemotherapies for glioblastoma multiforme (GBM). The present study exploits albumin as drug delivery vehicle to promote the chemotherapeutic efficacy of paclitaxel (PTX) by improving the stability and targeting efficiency of PTX/albumin nanoparticles (NPs). Here we characterize PTX-loaded human serum albumin (HSA) NPs stabilized with intramolecular disulfide bonds and modified with substance P (SP) peptide as the targeting ligand. The fabricated SP-HSA-PTX NPs exhibited satisfactory drug-loading content (7.89%) and entrapment efficiency (85.7%) with a spherical structure (about 150 nm) and zeta potential of −12.0 mV. The *in vitro* drug release from SP-HSA-PTX NPs occurred in a redox-responsive manner. Due to the targeting effect of the SP peptide, cellular uptake of SP-HSA-PTX NPs into brain capillary endothelial cells (BCECs) and U87 cells was greatly improved. The low IC_{50}, prolonged survival period and the obvious pro-apoptotic effect shown by TUNEL analysis

Abbreviations: BBB, blood–brain barrier; BBTB, blood–brain tumor barrier; BCECs, brain capillary endothelial cells; Cou-6, coumarin-6; D_2O, deuterium oxide; DDS, drug delivery system; DHO, deuterium hydrogen oxide; DLS, dynamic light scattering; EE, entrapment efficiency; FACS, fluorescence-activated cell sorting; GBM, glioblastoma multiforme; gp60, glycoprotein 60; GSH, glutathione; HPLC, high performance liquid chromatography; HSA, human serum albumin; MAL-PEG-NHS, maleimide-polyethylene glycol-ω-succinimidyl carbonate; MTT, [4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; NK-1, neurokinin-1; NPs, nanoparticles; PBS, phosphate-buffered saline; PhAsO, phenylarine oxide; PI, propidium iodide; PTX, paclitaxel; SP, substance P; SPARC, secreted protein acidic and rich in cysteine; TEM, transmission electron microscope

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all demonstrated that the fabricated SP-HSA-PTX NPs showed a satisfactory anti-tumor effect and could serve as a novel strategy for GBM treatment.

1. Introduction

Glioblastoma multiforme (GBM) is a kind of aggressive malignant brain cancer with the hallmark characteristics of uncontrolled cell proliferation and diffuse infiltration as well as the fatal prognosis with the median survival of less than 2 years[1,2]. Surgical methods still play the dominant role among the multiple approaches of GBM therapy, in which the bulk of the tumor is removed, but disappointingly, some peripheral parts are difficult to eradicate, leading often to relapse and metastasis[3]. Thus chemotherapy, a kind of noninvasive auxiliary treatment, remains indispensable for GBM patients. However, the blood–brain barrier (BBB) and blood–brain tumor barrier (BBTB) always serve as a formidable barrier to GBM treatments and severely limit the access of therapeutic agents to tumor cells within the brain[4]. Furthermore, the poor solubility, nonspecific distribution, rapid clearance and systemic toxicity of most chemotherapeutic drugs dramatically limits their applications, compromising the treatment efficiency and leading to severe adverse effects.

Recently, nanoparticulate drug delivery systems (DDS) have become a mature technical tool due to their optimized size and functionalized surface characteristics. The multifarious nanovehicles can package an existing drug to form various NPs which may improve the drug *in vivo* pharmacokinetic profiles, enhance drug accumulation at the targeted sites and thus further optimize therapeutic efficacy while avoiding toxic effects in healthy tissues[5]. However, the fact that the number of FDA approved products are much fewer than the preparations of the published achievements tells us that many challenges still remain in the optimization of the DDS from *in vitro* to *in vivo* translation, including lack of tolerability or high toxicity in patients as evidenced by the recall of many DDS formulations after commercialization[6]. Abraxane, one of the successfully marketed chemotherapy stars approved by the FDA in 2005 for the treatment of metastatic breast cancer, non-small cell lung cancer and pancreatic cancer[7] suggested to us that biomimetic materials originating from endogenous substances could be a favorable option with good biocompatibility and biodegradability. Thus, during the last decade we have witnessed an emerging paradigm shift to biomimetic materials due to their capacity to circumvent biological delivery barriers, along with increased specificity and compatibility with biological systems[8].

Among these biomimetics, albumin (also the vehicle used in Abraxane), an abundant and stable protein with a long circulatory half-life *in vivo*, has been explored as a versatile medical device for therapeutic and diagnostic agents due to its non-immunogenicity, inherent binding capacity for various drug molecules and targeting ability to malignant tissues by interacting with albumin-binding proteins, such as secreted protein acidic and rich in cysteine (SPARC) and glycoprotein 60 (gp60)[9–11]. Nevertheless, one of the obvious issues of an albumin-based drug delivery system is poor structural stability due to their native characteristics and the complex *in vivo* environment full of proteins and enzymes[12]. In terms of Abraxane, PTX molecules are not covalently linked to albumin, resulting in a poor stability in the circulation and unexpected PTX-leak before reaching the targets. Besides the tumor tissues, PTX is easily distributed into healthy organs, which will lead to wide systemic toxicity[13].

Inspired by the application of cross-linkers in polymeric micelles[14], we attempted to introduce a covalent linkage between the albumin molecules to improve physical adsorption between drugs and albumin. The abundant amino acids on albumin molecules contain 17 pairs of disulfide bonds that could be cleaved by reducing reagents. In an oxidative atmosphere intermolecular disulfide bonds can be reformed, leading to a re-assembly of the albumin molecules into relatively stable NPs and an improved encapsulating capability of drugs into the hydrophobic domain[15]. It is known that the level of glutathione (GSH), which can cleave disulfide bonds in tumor cells (10 mmol/L), is much higher than that in normal cells (0.2 mmol/L), which suggests that the intermolecular disulfide bonds between albumin molecules could be selected as an ideal self-cross-linker to realize preferred stabilization strategy of drugs as well as redox-responsive release in tumor cells[16,17].

Besides excellent *in vivo* stability and biocompatibility, efficient delivery of drugs is also necessary for a smart delivery system. The original uptake mechanism of albumin-based NPs is mainly mediated by SPARC and gp60 receptor. However, it is not enough to realize a favorable targeting effect in a complicated tumor microenvironment. Thus, some researchers have modified albumin NPs with various targeting moieties such as cetuximab[18], glycyrrhetinic acid[19] and cyclic Arg-Gly-Asp (RGD)[20] to achieve active targeting ability and promote drug accumulation in tumors. To endow the albumin NPs with passive targeting as well as active targeting ability to cross the BBB and BBTB effectively for the treatment of GBM, neurokinin-1 (NK-1) receptors are found to be selectively overexpressed in several malignant tumors including glioma[21]. SP peptide (with a sequence as Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met) one of the NK-1 binding ligands, could be exploited as a targeting ligand on albumin NPs easily *via* a PEG linker.

In this study, we developed a glioma-targeting drug delivery system based on biomimetic albumin material with good stability and favorable biosafety. We first fabricated a stable human serum albumin (HSA) nanoparticle loaded with PTX (HSA-PTX NPs) with a redox-responsive characteristic and then SP peptide was covalently anchored on the HSA-PTX NPs (SP-HSA-PTX NPs) to improve tumor accumulation of PTX at the glioma site. The physicochemical properties of SP-HSA-PTX NPs, including particle size, surface morphology, drug loading and redox-responsive behaviors were studied in detail. Furthermore, the *in vitro* and *in vivo* therapeutic efficacy of the NPs was investigated in BCECs and U87 cells. Hopefully, this study will cast a new light for the biomimetic platform in tumor targeting delivery.

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