Biomaterials 32 (2011) 8010–8020

Contents lists available at ScienceDirect

Biomaterials

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

Aptamer-functionalized PEG–PLGA nanoparticles for enhanced anti-glioma drug delivery

Jianwei Guo [a,c,1], Xiaoling Gao [b,1], Lina Su [a,c], Huimin Xia [a], Guangzhi Gu [a], Zhiqing Pang [a], Xinguo Jiang [a], Lei Yao [b], Jun Chen [a,*], Hongzhuan Chen [b,*]

[a] Department of Pharmaceutics, Key Laboratory of Smart Drug Delivery, Ministry of Education & PLA, School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, PR China
[b] Department of Pharmacology, Institute of Medical Sciences, Shanghai Jiaotong University School of Medicine, 280 South Chongqing Road, Shanghai 200025, PR China
[c] College of Pharmacy, Dali University, Wanhua Road, Xiaguan City, Yunnan 671000, PR China

ARTICLE INFO

Article history:
Received 8 May 2011
Accepted 4 July 2011
Available online 23 July 2011

Keywords:
Aptamer
Nucleolin
Nanoparticle
Chemotherapy
Paclitaxel
Drug delivery

ABSTRACT

Targeted delivery of therapeutic nanoparticles in a disease-specific manner represents a potentially powerful technology especially when treating infiltrative brain tumors such as gliomas. We developed a nanoparticulate drug delivery system decorated with AS1411 (Ap), a DNA aptamer specifically binding to nucleolin which was highly expressed in the plasma membrane of both cancer cells and endothelial cells in angiogenic blood vessels, as the targeting ligand to facilitate anti-glioma delivery of paclitaxel (PTX). Ap was conjugated to the surface of PEG–PLGA nanoparticles (NP) via an EDC/NHS technique. With the conjugation confirmed by Urea PAGE and XPS, the resulting Ap-PTX-NP was uniformly round with particle size at 156.0 ± 54.8 nm and zeta potential at −32.93 ± 3.1 mV. Ap-nucleolin interaction significantly enhanced cellular association of nanoparticles in C6 glioma cells, and increased the cytotoxicity of its payload. Prolonged circulation and enhanced PTX accumulation at the tumor site was achieved for Ap-PTX-NP, which eventually obtained significantly higher tumor inhibition on mice bearing C6 glioma xenografts and prolonged animal survival on rats bearing intracranial C6 gliomas when compared with PTX-NP and Taxol®. The results of this contribution demonstrated the potential utility of AS1411-functionalized nanoparticles for a therapeutic application in the treatment of gliomas.

1. Introduction

Brain tumors remain a significant health problem worldwide, among which glioma is the most commonly diagnosed one, accounting for approximately 45%–50% of all primary brain tumors [1,2]. As an aggressive malignant form of cancer, glioma often results in death of affected patients within one to two years following diagnosis. A defining feature of high-grade glioma (and also seen in lower grade gliomas) is that the tumor does not have a sharp border, individual tumor cells infiltrate the brain, and are likely to be widely distributed at the time of diagnosis [3]. Therefore, malignant brain glioma can rarely be cured with only surgery and radiotherapy. Chemotherapy seems essential in the auxiliary treatment of malignant glioma. However, although has been demonstrated to provide a survival benefit to high-grade glioma patients, the results of chemotherapy have been modest at best [4,5]. Explanations for the poor results include the non-specific, non-targeted nature of most of the drugs currently used and their inadequate delivery to the tumor.

Nanobiotechnology, particularly nanoparticles, is making a significant contribution to the improvement of drug delivery in cancer and many of these technologies can be applied to glioma. The challenge lies in the design of nanoparticles (NPs) that are able to overcome the blood–brain/blood–tumor barrier, specifically and differentially taken up by glioma cells and release their payload over an extended period to achieve a clinical response. NPs derived from poly (D,L-lactic-co-glycolic acid) (PLGA) as the controlled release polymer system are an excellent choice since their safety in clinic is well established [6]. Poly(ethylene glycol) (PEG)-functionalized PLGA NPs are especially desirable because pegylated polymeric NPs have significantly reduced systemic clearance compared with similar particles without PEG [7,8], which is especially important for the passive targeting of nanocarrier to tumor by the enhanced permeability and retention (EPR) effects. The development of biotechnology has provided targeting ligands that

* Corresponding authors. Tel.: +86 21 51980066; fax: +86 (0) 21 51980069.
E-mail addresses: chenjun@fudan.edu.cn (J. Chen), hongzhuan_chen@hotmail.com (H. Chen).
[1] Authors contributed equally.

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.07.004

specifically bind to biologically active molecules or receptors highly expressed on blood–brain/blood–glioma barrier or glioma cells, which further enabled active delivery of chemotherapeutic agents to gliomas [9,10].

Aptamers are single-stranded DNA or RNA oligonucleotides that fold into specific 3D structures, and bind to target molecules with high specificity and affinity. Because of their low molecular weights, lack of immunogenicity, and readily availability, aptamers are good candidates for targeted imaging and therapy. Various aptamers have been developed against a variety of cancer targets, including extracellular ligands and cell surface proteins [11]. AS1411 is a DNA aptamer that binds to nucleolin, a protein highly expressed in the plasma membrane of cancer cells [12], and has been successfully exploited as a targeting ligand for tracking C6 glioma cells [13,14]. It has also been pointed out that nucleolin is highly expressed at the cell surface of endothelial cells in the angiogenic blood vessels [15,16], and participates in binding and endocytosis/macropinocytosis, processes with potential applications in drug delivery [17,18]. Therefore, we speculated that the AS1411-nucleolin interaction could be utilized as a strategy to mediate highly specific and effective drug delivery to gliomas.

Paclitaxel (PTX), a widely used chemotherapeutic agent isolated from the bark of *Taxus brevifolia*, showed anti-neoplasic activity against various types of solid tumors such as ovarian, breast and lung cancers [19,20], and has also been proven effective in the treatment of gliomas [21–24]. However, its clinical efficacy is often compromised by its poor aqueous solubility, non-tumor-specific cell-killing and serious adverse effects induced by its solvent—Cremophor EL-ethanol [25].

In this study, in order to improve the anti-glioma efficacy of paclitaxel, an AS1411 aptamer-functionalized nanoparticulate drug delivery system (Ap-PTX-NP) was developed. PTX-loaded nanoparticles (PTX-NP) were prepared via an emulsion/solvent evaporation method using PLGA–PEG–COOH amphiphilic copolymer as the matrix. AS1411 was conjugated to PTX-NP surface through an EDC/NHS technique (Fig. 1). The Ap modification was confirmed by urea polyacrylamide gel electrophoresis (urea PAGE) and X-ray photoelectron spectroscopy (XPS). The resulting nanoparticles were characterized in terms of particle size, zeta potential, surface morphology, drug encapsulation efficiency (EE), drug loading capacity (LC) and *in vitro* drug release. Cellular association of Ap-NP and anti-proliferation effect of Ap-PTX-NP was evaluated on C6 glioma cells. Pharmacokinetic and biodistribution were performed to determine the tumor targeting properties of Ap-PTX-NP. *In vivo* tumor growth inhibition and survival experiment was performed on mice bearing C6 glioma xenografts and rats bearing intracranial C6 gliomas, respectively, to evaluate its anti-glioma efficacy.

2. Materials and methods

2.1. Materials

PTX was purchased from Xi'an Sanjiang Biological Engineering Co. Ltd, and Taxol® from Bristol–Myers Squibb Company. Poly (D,L-lactide-co-glycolide) (50/50) with terminal carboxylate groups (PLGA, inherent viscosity 0.18 dl/g in hexafluoroisopropanol, MW 15 kDa) was obtained from PURCA (Holland). NH_2–PEG–COOH (MW 3500) was purchased from JenKem technology Co. LTD (Beijing, China). AS1411 DNA aptamer (Ap, sequence: 5′-TTGGTGGTGGTG GTTGTGGTGGTGGTGG-3′, 28 bp) was custom synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Both 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and coumarin 6 were provided by Sigma–Aldrich (St. Louis, MO, USA). Dubelcco's Modified Eagle's Medium (DMEM), trypsin–EDTA and penicillin–streptomycin solution were provided by Invitrogen (Merelbeke, Belgium), and fetal bovine serum (FBS) by Gibco BRL (Carlsbad, CA, USA). Double distilled water was purified using a Millipore Simplicity System (Millipore, Bedford, USA). All other reagents were of analytical or chromatographic pure grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Rat C6 glioma cell line was purchased from Cell Institute of Chinese Academy of Sciences (Shanghai, China).

Sprague–Dawley (SD) rats, Wistar rats and nude mice were obtained from the Shanghai Laboratory Animal Resources Center (Shanghai, China) and treated according to the protocols evaluated and approved by the ethical committee of Fudan University.

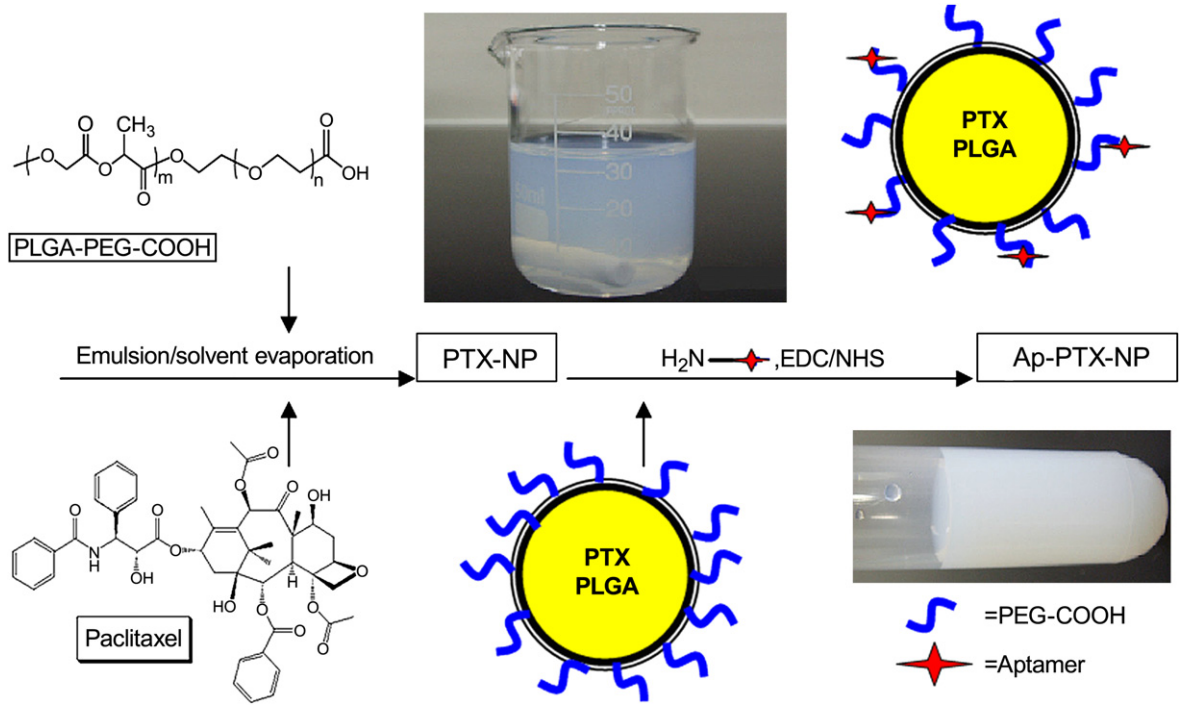

Fig. 1. Preparation of aptamer-functionalized paclitaxel-loaded nanoparticles (Ap-PTX-NP). PTX was encapsulated in the PLGA–PEG–COOH nanoparticle via an emulsion/solvent evaporation method. The nanoparticles were decorated with Ap by covalently conjugating amine-terminated Ap to carboxylate-functionalized PTX-NP surface through an EDC/NHS technique. EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; NHS, N-hydroxysuccinimide.