Biomaterials 33 (2012) 8167–8176

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Anti-glioblastoma efficacy and safety of paclitaxel-loading Angiopep-conjugated dual targeting PEG-PCL nanoparticles

Hongliang Xin [a,b], Xianyi Sha [a], Xinyi Jiang [a], Wei Zhang [a], Liangcen Chen [a], Xiaoling Fang [a,*]

[a] Key Laboratory of Smart Drug Delivery, Ministry of Education & PLA, School of Pharmacy, Fudan University, Lane 826, Zhangheng Road, Shanghai 201203, China
[b] Department of Pharmaceutics, School of Pharmacy, Nanjing Medical University, Nanjing 210029, China

ARTICLE INFO

Article history:
Received 25 May 2012
Accepted 23 July 2012
Available online 11 August 2012

Keywords:
Dual target strategy
Nanoparticles
Brain tumor
Glioma penetration
Anti-glioblastoma
Toxicity

ABSTRACT

Therapeutic effect of glioma is often limited due to low permeability of delivery systems across the Blood-Brain Barrier (BBB) and poor penetration into the tumor tissue. In order to overcome the two barriers, we proposed Angiopep-conjugated PEG-PCL nanoparticles (ANG-PEG-NP) as a dual targeting drug delivery system for glioma treatment basing on low density lipoprotein receptor related protein (LRP) receptor not only over-expressed on BBB but also on glioma cells. This system could transport across BBB through LRP-mediated transcytosis and then targeted glioma via LRP-mediated endocytosis. In this study, we evaluated the preliminary availability and safety of ANG-PEG-NP for glioma treatment. The penetration, distribution, and accumulation into 3D glioma spheroid and *in vivo* glioma region of ANG-PEG-NP were obviously higher than that of plain PEG-PCL nanoparticles (PEG-NP). The anti-glioblastoma efficacy of paclitaxel (PTX) loading ANG-PEG-NP was significantly enhanced as compared to that of Taxol and PEG-NP. Preliminary safety results showed that no acute toxicity to hematological system, liver, kidney and brain tissue was observed after intravenous administration with a dose of 100 mg/kg blank ANG-PEG-NP per day for a week. Results indicate that Angiopep-conjugated dual targeting PEG-PCL nanoparticle is a potential brain targeting drug delivery system for glioma treatment.

1. Introduction

At present, glioblastoma multiforme (GBM) is the most frequent primary central nervous system tumor in human. Although the advances in other solid tumor therapy have improved the survival of patients, GBM prognosis is still poor, with a 14-month median survival time despite interventions [1]. Because GBM is different from other cancers by its diffuse invasion of the surrounding normal brain tissue, it is impossible to make the complete removal of glioma by conventional surgery and the chances of glioma recurrence from residual tumors are very high [2]. Therefore, chemotherapy is indispensable for glioma treatment after the surgery [3]. Unfortunately, the GMB treatment by chemotherapy is very limited due to the rare blood brain barrier (BBB) penetration and poor glioma targeting of the chemotherapeutics [4]. Although compromised endothelial barrier which facilitates molecular transport under glioma condition exists [5,6], BBB still plays important role in the infiltrating margin of glioma and should be considered for glioma treatment and diagnosis [7]. Paclitaxel (PTX) is a new class of microtubule stabilizing agents with perfect anti-glioma activity [8,9]. However, the activity of commercial PTX preparation against glioblastoma has been disappointing in clinical study because of drug-resistance and poor penetration across the BBB [10,11]. Accordingly, it is impending to develop a targeted drug delivery system with high BBB penetration and glioma targeting abilities.

To overcome BBB and blood tumor barrier (BTB) [12,13], dual targeting drug delivery systems based on receptor-mediated endocytosis were developed to deliver chemotherapeutic agent across BBB and simultaneously target brain tumor [14]. The most common dual targeting strategy is based on modification of nanocarriers with two kinds of ligands, one of which can target to BBB, the other can target to glioma cancerous cells [15,16]. It has been reported that low density lipoprotein receptor related protein (LRP) is not only over-expressed on BBB but also on glioma cancerous cells. Thus, another dual targeting strategy was developed to decorate the surface of nanocarriers with one single ligand [17]. In our previous study, Angiopep-2, a specific ligand of LRP receptor, was used to modify poly(-ethylene glycol)-co-poly(ε-caprolactone) (PEG-PCL) copolymer nanoparticles to develop a dual targeting drug delivery system (ANG-PEG-NP) for PTX delivery [17]. The glioma dual targeting

* Corresponding author. Tel.: +86 21 51980071; fax: +86 21 51980072.
E-mail address: xlfang@shmu.edu.cn (X. Fang).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.07.046

strategy of PTX loading Angiopep-conjugated PEG-PCL nanoparticles (ANG-PEG-NP-PTX) was shown as Fig. 1, which denoted that Angiopep-2 mediated transcytosis of ANG-PEG-NP-PTX across BBB through LRP as grade I targeting, followed by endocytosis of ANG-PEG-NP-PTX via recognition of LRP on the surface of glioma cells as grade II targeting. The *in vitro* and *in vivo* brain targeting mechanism [18] and enhancement of cytotoxicity to U87 MG glioma cells [17] of ANG-PEG-NP were also confirmed in our previous studies. However, there are still several challenges need to be addressed: firstly, since the solid tumor microenvironment which contains clusters of tumor cells, nonuniform leaky vasculature and a dense interstitial structure differs from *in vitro* cancerous cells by its structural heterogeneity [19], so can ANG-PEG-NP enhance the penetration, distribution, and accumulation of chemotherapeutic agent in the solid tumor *in vivo*? Secondly, what will be the difference between targeted and non-targeted nanoparticles in anti-glioblastoma efficacy assessment using intracranial glioma mice model? Thirdly, considering part of ANG-PEG-NP will accumulate in the brain parenchyma and other organ tissues, can this accumulation of dual targeting nanoparticles induce functional disorder and other toxicities?

In this study, we used *ex vivo* 3D glioma tumor spheroids and intracranial glioma mice model to evaluate the penetration, distribution, and accumulation of ANG-PEG-NP into brain tumor. The *in vivo* anti-glioblastoma efficacy of ANG-PEG-NP was investigated by intracranial glioma mice model as well. The safety of ANG-PEG-NP following intravenous injection was carried out using healthy mice.

2. Materials and methonds

2.1. Materials

Methoxyl poly(ethylene glycol)-co-poly(ε-caprolactone) copolymer (Me-PEG–PCL, 12 KDa) and Maleimidyl-poly(ethylene glycol)-co-poly(ε-caprolactone) copolymer (Maleimide-PEG-PCL, 14 KDa) were synthesized by the ring opening polymerization as described before [17]. Rhodamine B isothiocyanate (RBITC), Propidium Iodide (PI), MTT were purchased from Sigma (St. Louis, MO, USA). Low melting-point agarose was obtained from Yixin Biotechnology Co., Ltd. (Shanghai, China). Angiopep (TFFYGGSRGKRNNFKTEEYC) was synthesized by Shanghai GenePharma Co., Ltd Company (Shanghai, China). Penicillin-streptomycin, DMEM, fetal bovine serum (FBS) and 0.25% (w/v) trypsin solution were purchased from Gibco BRL (Gaithersberg, MD, USA). BCA kit and TritonX-100 were purchased from Beyotime Biotechnology Co., Ltd. (Nantong, China). All the other solvents were analytical grade.

2.2. Cell line

The U87 MG cell line was obtained from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Brain capillary endothelial cells (BCECs) were kindly provided by Prof. X. G. Jiang (School of Pharmacy, Fudan University). Both cell lines were cultured in DMEM medium, supplemented with 10% FBS, 1% nonessential amino acids, 100 IU/mL penicillin and 100 μg/mL streptomycin sulfate. Cells were cultured in incubators maintained at 37 °C with 5% CO_2 under fully humidified conditions. All experiments were performed in the logarithmic phase of cell growth.

2.3. Animals

Male BALB/c nude mice and ICR mice, aging 4–5 weeks and weighing 20 ± 2 g, were supplied by Department of Experimental Animals, Fudan University (Shanghai, China). Mice were acclimated at 25 °C and 55% of humidity under natural light/dark conditions for 1 week before animal study. All animal experiments were carried out in accordance with the guidelines approved by the ethics committee of Fudan University (Shanghai, China).

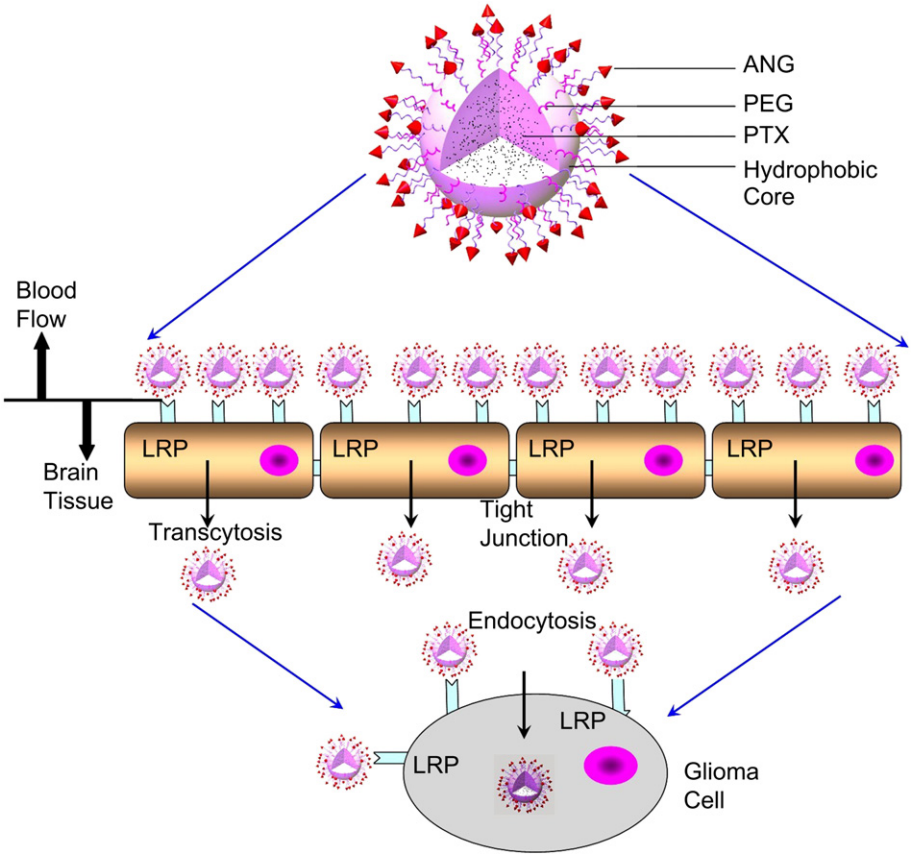

Fig. 1. Design of PTX loading Angiopep-conjugated polymer nanoparticles as dual targeting drug delivery system for glioma via LRP mediated endocytosis.