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Paclitaxel-loaded solid lipid nanoparticles modified with Tyr-3-octreotide for enhanced anti-angiogenic and anti-glioma therapy

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ABSTRACT

Somatostatin receptors (SSTRs) especially subtype 2 (SSTR2) are overexpressed in glioma. By taking advantage of the specific expression of SSTR2 on both glioma neovasculature endothelial cells and glioma cells, we constructed Tyr-3-octreotide (TOC)-modified solid lipid nanoparticles (SLN) loaded with paclitaxel (PTX) to enable tumor neovasculature and tumor cells dual-targeting chemotherapy. In this work, a TOC-polyethylene glycol-lipid (TOC-PEG-lipid) was successfully synthesized and used as a targeting molecule to enhance anticancer efficacy of PTX loaded sterically stabilized lipid nanoparticles. The prepared PTX-loaded SLN modified with TOC (PSM) was characterized by standard methods. In rat C6 glioma cells, PSM improved PTX induced apoptosis. Both tube formation assay and CD31 staining of treated orthotopic glioma tissues confirmed that PSM significantly improved the antiangiogenic ability of PTX in vitro and in vivo, respectively. Radiolabelled PSM achieved a much higher and specific accumulation within the glioma as suggested by the biodistribution and imaging studies. Furthermore, PSM exhibited improved anti-glioma efficacy over unmodified nanoparticles and Taxol in both subcutaneous and orthotopic tumor models. These findings collectively indicate that PSM holds great potential in improving the efficacy of anti-glioma therapy.

Statement of Significance

Somatostatin receptors (SSTRs) especially subtype 2 (SSTR2) are overexpressed in various mammalian cancer cells. Proliferating endothelial cells of neovasculature also express SSTR2. Tyr-3-octreotide (TOC) is a known ligand for SSTR2. We have successfully prepared paclitaxel-loaded solid lipid nanoparticles modified with TOC (PSM) having diameter less than 100 nm. We found that PSM improved anticancer efficacy of paclitaxel in SSTR2 positive glioma of rats. This improved anti-glioma efficiency of PSM can be attributed to dual-targeting (i.e. tumor cell and neovasculature targeting) efficiency of PSM and promoted anti-cancer drug accumulation at tumor site due to TOC modification of solid lipid nanoparticles. This particular study aims at widening the scope of octreotide-derivative modified nanocarrier by exploring dual-targeting potential of PSM.

1. Introduction

Glioma is one of the deadliest diseases in this world. Treatment of glioma is one of the most challenging problems in neuro-oncology, and patients diagnosed with glioma have a

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median survival of less than 2 years. Current therapies of glioma include combination of surgery, radiotherapy and chemotherapy. Chemotherapy has been considered as the most common adjuvant therapy for glioma [1]. However, non-targeted nature of anti-cancer drugs and several physiologic barriers (e.g. blood-brain barrier) restrict chemotherapy to achieve its full potential. To overcome these brain delivery problems associated with conventional anti-cancer drugs, the lipid nanoparticulate delivery system can be an attractive choice. It has been reported in the literature that the use of solid lipid nanoparticles (SLN) is advantageous due to lower cytotoxicity, higher drug loading capacity, and best production scalability [2]. In this work, we have used PTX as a model chemotherapeutic drug for loading into SLN.

PTX is effective against various types of cancers, including gliomas [3]. A major drawback in PTX chemotherapy is that PTX has very low solubility in water and many pharmaceutically acceptable solvents. Till date, two of the formulations of PTX namely Taxol and Abraxane are approved by the Food and Drug Administration (FDA), USA. Taxol formulation contains PTX dissolved in a 50:50 (v/v) mixture of Cremophor EL and dehydrated alcohol; however, serious side effects such as hypersensitivity, nephrotoxicity and neurotoxicity have been reported for Taxol mainly due to the presence of Cremophor EL. On the other hand, Abraxane, an injectable suspension of albumin-bound PTX nanoparticles, is approved for use in patients with breast cancer, non-small cell lung cancer and metastatic pancreatic cancer [4,5]. However, there is no evidence that Abraxane has the activity against brain tumors. In this report, we evaluated the anti-glioma and anti-angiogenic potential of TOC-modified PTX-loaded SLN.

Angiogenesis is an integral hallmark of cancer [6]. Several agents that inhibit tumor angiogenesis have been successfully translated into the clinic. Gliomas are rich in vascular endothelial growth factor (VEGF), which promotes angiogenesis. In recent years, Anti-angiogenic therapy has come to the forefront as an alternative to other glioma treatment strategies. However, new strategies are still urgently required to overcome the poor response and resistance in some antiangiogenic therapies. Tumor neovasculature and tumor cells dual-targeting chemotherapy offers an alternative option and may provide a more promising therapeutic strategy.

Effective tumor neovasculature and tumor cells dual-targeting depends on the identification of high-quality biomarkers of pathology [7]. The overexpression of SSTRs on various tumor cells provides the molecular basis for the successful use of radiolabelled somatostatin analogues (e.g. different octreotide derivatives) with higher stability than the parent molecule somatostatin. It has been reported in the literature that gliomas constantly overexpress the receptor subtype SSTR2 [8]. Apart from that SSTR2 expression has also been found on endothelial cells of proliferating vessels of gliomas [9]. Therefore, targeting the SSTR2 may provide enhanced efficacy of chemotherapeutic-loaded SLN against glioma.

Octreotide and TOC-modified nanocarriers have been reported for SSTR2 positive tumor targeting [10,11]. However, tumor cell and tumor neovasculature dual-targeting potential of these nanocarriers in SSTR2 positive glioma has not been evaluated. Besides, TOC-modified nanocarrier has not been used for drug delivery purpose [11]. It has also been shown that binding affinity and % uptake of radiolabelled TOC in SSTR2 positive tumors are higher than radiolabelled octreotide (OC) [12]. With this potential of TOC we hypothesized that TOC modification of sterically stabilized SLN may improve anti-glioma efficacy of PTX. In this study, a TOC-PEG-lipid (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) was used as lipid) was synthesized and used to construct PSM. We evaluated the antitumor efficacy in C6 rat glioma as it is a very reliable model for human glioma growth and invasion [13].

2. Materials and methods

2.1. Reagents, cell culture and animals

2.1.1. Reagents

PTX was obtained as a gift sample from Fresenius Kabi Oncology Ltd., Kalyani, India. $^{99}Mo/^{99m}Tc$ kits were obtained from Board of Radiation and Isotope Technology (BRIT), Mumbai, India. All other reagents and solvents were obtained from Sigma-Aldrich, St. Louis, USA or Merck, Hohenbrunn, Germany or from SRL, Mumbai, India, and they are either high performance liquid chromatography (HPLC) or analytical grade. All chemicals were used without further purification. All experiments were carried out at room temperature (25 ± 2 °C) unless otherwise mentioned.

2.1.2. Cell culture

Rat C6 glioma cell line and NIH 3T3 fibroblast cell line were obtained from National Centre for Cell Science, Pune, India. Cells were grown confluent in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin–streptomycin (Pen-Strep) (100 IU ml^{-1}–100 μg ml^{-1}), and 1 mM glutamine at 37 °C in a humid atmosphere (5% CO_2–95% air). The cells were subcultured every 2–3 days by employing a trypsin/EDTA (0.05/0.02% w/v) solution. Only the cells showing viability >97% were used for the experiments. The cells for all experiments were in the logarithmic phase of growth. All reagents for cell culture were purchased from Invitrogen-Gibco, UK.

2.1.3. Animals

Animal experiments were performed in compliance with the regulations of the Institutional Animal Ethics Committee, CSIR-IICB, Kolkata, India. In-house female Sprague-Dawley (S/D) rats were used for all animal studies. To develop subcutaneous tumors, we used 4-week-old S/D rats; whereas we used adult (~6 months age) S/D rats to develop orthotopic models. All rats were housed on a standard laboratory diet at an ambient temperature and humidity in air-conditioned chambers.

2.2. Synthesis and characterization of TOC-PEG-DPPE (TPD)

2.2.1. Synthesis of TOC

TOC was synthesized by following a standard Fmoc solid phase synthesis [14] on O-t-butyl-threoninol-2-chlorotrityl resin [substitution 0.67 mmol/g, 0.1 mmol (i.e. ~149.3 mg) swelled in N,N-Dimethylformamide (DMF)]. After synthesis of octapeptide resin, a small fraction was cyclised, cleaved from the resin, and purified by HPLC as described previously [14]. Matrix-assisted laser desorption/ionization (MALDI) mass spectra of purified TOC were recorded on an Applied Biosystems 4700 proteomics analyzer 170. Apart from that ^1H nuclear magnetic resonance (NMR) analysis of pure TOC in D_2O was also performed. ^1H NMR spectra were acquired with a Bruker DRX 600 MHz NMR instrument at ambient temperature.

2.2.2. Synthesis of TOC-PEG

Conjugation of PEG 600 diacid (10 equivalent) to the octapeptide-resin was done in DMF with N,N′-Diisopropylcarbodiimide (DIPCDI) (20 equivalent) and 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate hexafluorophosphate (TBTU) (10 equivalent) for 5 h by nitrogen bubbling. Coupling of PEG 600 diacid was monitored by 2,4,6-Trinitrobenzenesulfonic acid (TNBS) test. After the synthesis of resin-octapeptide-PEG-acid, a small fraction was cyclised, cleaved from the resin, and purified by HPLC [14]. After purification, the compound was given for MALDI mass analysis for confirmation of synthesis of TOC-PEG.

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