Somatostatin Receptor-Mediated Specific Delivery of Paclitaxel Prodrugs for Efficient Cancer Therapy

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ABSTRACT: In this study, a novel PTX prodrug, octreotide(Phe)–polyethene glycol–paclitaxel [OCT(Phe)–PEG–PTX], was successfully synthesized and used for targeted cancer therapy. A nontargeting conjugate, mPEG–PTX, was also synthesized and used as a control. Chemical structures of OCT(Phe)–PEG–PTX and mPEG–PTX were confirmed using ^{1}H nuclear magnetic resonance and circular dichroism. The drug contents in both the conjugates were 12.0% and 14.0%, respectively. Compared with the parent drug (PTX), OCT(Phe)–PEG–PTX, and mPEG–PTX prodrugs showed a 20,000- and 30,000-fold increase in water solubility, respectively. PTX release from mPEG–PTX and OCT(Phe)–PEG–PTX exhibited a pH-dependent profile. Moreover, compared with mPEG–PTX, OCT(Phe)–PEG–PTX exhibited significantly stronger cytotoxicity against NCI-H446 cells (SSTR overexpression) but comparable cytotoxicity against WI-38 cells (no SSTR expression). Results of confocal laser scanning microscopy revealed that the targeting prodrug labeled with fluorescence probe was selectively taken into tumor cells via SSTR-mediated endocytosis. *In vivo* investigation of prodrugs in nude mice bearing NCI-H446 cancer xenografts confirmed that OCT(Phe)–PEG–PTX prodrug exhibited stronger antitumor efficacy and lower systemic toxicity than mPEG–PTX and commercial Taxol. These results suggested that OCT(Phe)–PEG–PTX is a promising anticancer drug delivery system for targeted cancer therapy. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2018–2028, 2015

Keywords: prodrugs; cancer chemotherapy; targeted drug delivery; peptide delivery; polymeric drugs

INTRODUCTION

Paclitaxel (PTX) exhibited a significant activity against various solid tumors because of its ability to promote tubulin assembly into microtubules.[1] However, serious drawbacks hamper the clinical application of PTX. First, PTX lacks selective cytotoxicity against cancer cells and frequently leads to serious side effects.[2] Poor water solubility of PTX (0.3 μg/mL) is another concern that significantly reduces its wider clinical application. To enhance its water solubility, PTX is currently formulated as a 50:50 mixture of Cremophor EL and ethanol (Taxol). However, the amount of Cremophor EL required to solubilize PTX is considerably high (26 mL Cremophor EL for a single intravenous administration in an average patient), which leads to significant side effects such as hypersensitivity, neurotoxicity, nephrotoxicity, and cardiotoxicity.[3]

The prodrug strategy is promising to overcome these problems.[4,5] Over the last decades, several studies have been conducted on prodrug synthesis of PTX. Poly(ethylene glycol) methyl ether methacrylate,[6] cyclotriphosphazene,[7] poly(vinyl alcohol),[8] and poly(L-glutamic acid)[9] have been proposed as PTX carriers. However, only a part of the prodrug can efficiently accumulate in the tumor site through the enhanced permeability and retention (EPR) effect, which decrease drug efficiency and increase its toxicity. Further improvement can be achieved by conjugating targeting ligands onto polymeric prodrugs to achieve selective delivery to tumor cells.[10] Receptor-targeted polymeric prodrugs have been shown to improve therapeutic responses both *in vitro* and *in vivo*.[11] Various ligands have been investigated, including folate,[12] transferrin,[13] antibodies,[14] peptides,[15] and aptamers.[16]

Somatostatin (SST) is a polypeptide that is released in the gastrointestinal tract by delta cells and the hypothalamus. It is a key regulatory peptide that inhibits hormones such as gastrin, cholecystokinin, glucagon, growth hormone, insulin, and secretin.[17] Cellular actions of SST are mediated by five SST receptors (SSTR 1–5). SSTRs are G-protein-coupled receptors that are widely distributed in various tumors, including small cell lung cancer, neuroendocrine tumors, prostate cancer, breast cancer, colorectal carcinoma, gastric cancer, and hepatocellular carcinoma.[18,19] Clinical usefulness of SST is limited by its very short half-life. Several synthetic SST analogs, including octreotide (OCT), lanreotide, and vapreotide, have improved metabolic stability and increased affinity to SSTRs.[20]

Octreotide, one of the most extensively studied SST analogs, selectively binds to SSTR2 and SSTR5. It is composed of eight amino acids H_2N-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol (disulfide bridge Cys2-Cys7). OCT has been used clinically to prevent carcinoid crisis[21] and to visualize tumors containing high density of SSTRs by using scintigraphy.[22] For example, radiolabeled OCT derivatives, ^{99m}Tc-OCT and ^{111}In-OCT, are very useful for detecting small neuroendocrine tumors that cannot be detected by conventional methods.[23,24] In addition to its successful application on radio-oncology, OCT is further used to enhance the delivery of drugs to tumor cells by modifying them on the surface of nanocarriers.[25,26] These promising results prompted us to develop OCT as a specific targeting moiety for delivering a polymeric prodrug of PTX into tumor cells via SSTR endocytosis.

Polyethylene glycol (PEG) modification of drugs, also called PEGylation, is widely performed to improve the solubility and *in vivo* stability of drugs.[27] Use of PEG is widespread because of its unique physicochemical characteristics such as low

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polydispersity in molecular weight and solubility in aqueous as well as many organic solvents.[28] Moreover, PEGylation prevents rapid renal clearance of drugs and decreases their uptake by mononuclear phagocyte system, thus prolonging their *in vivo* half-life.[29] In addition, passive tumor targeting of drugs can be achieved by their PEGylation through the EPR effect.[30]

In this study, a novel polymeric prodrug system of OCT(Phe)–PEG–PTX was developed for targeted tumor therapy. Targeting ligand OCT helps in achieving high local concentrations of antitumor drugs to improve therapeutic efficiency, and water-soluble PEGs improve water solubility of parent drugs and improve the pharmacokinetic profile of PTX while reducing its side effects. Nontargeted prodrug mPEG–PTX was prepared as a control. The present study investigated the aqueous solubilities of OCT(Phe)–PEG–PTX and mPEG–PTX and *in vitro* release profiles of parent drugs from polymeric drugs. Then, the cytotoxicity, cellular uptake, and intracellular distribution of prodrugs were evaluated on human nonsmall lung cancer cells (NCI-H446; SSTR overexpression) and human embryonic lung fibroblasts (WI-38 cells, no SSTRs expression). Finally, *in vivo* antitumor efficacy and systemic toxicity of OCT(Phe)–PEG–PTX on nude mice bearing NCI-H446 cancer xenografts were compared with those of nontargeting mPEG–PTX and Taxol formulation.

MATERIALS AND METHODS

Materials

Paclitaxel was obtained from Chongqing Meilian Pharmaceutical Company, Ltd. (Chongqing, China). OCT (molecular weight, 1019.26 Da) was kindly provided by Shanghai Soho-Yiming Pharmaceuticals Company, Ltd. (Shanghai, China). Succinic anhydride (SA) was purchased from Shanghai LingFeng Reagent Company, Ltd. (Shanghai, China), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloric acid salt (EDC·HCl) was purchased from GL Biochem (Shanghai, China). Methoxypolyethylene glycol amine (mPEG-NH_2; molecular weight, 5000 Da) and *N*-hydroxysuccinimide (NHS) were purchased from Aladdin Reagent Company Ltd. (Shanghai, China). Succinimidyl carboxymethyl ester (SCM, Boc-NH-PEG-NHS) was obtained from Creative PEGWorks (Winston-Salem, North Caroline), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Company (St. Louis, Missouri). Cremophor EL was kindly gifted by BASF Corporation (Ludwigshafen, Germany). All other reagents were of analytical grade and were used without further purification. Distilled and deionized (DI) water was used in all experiments.

Methods

Synthesis of OCT(Phe)–PEG–PTX and mPEG–PTX Prodrugs

Octreotide(Phe)–PEG–PTX and mPEG–PTX were synthesized using a three-step procedure, according to the general scheme presented in Scheme 1.

Synthesis of 2′-O-Succinyl-Paclitaxel

2′-*O*-Succinyl-Paclitaxel (2′-SA-PTX) was synthesized as previously reported.[31] The crude product was purified by silica gel column chromatography with ethyl acetate–*n*-hexane–ethylic acid (6:1:0.1) as eluent to give the intermediate (yield = 90.8%) for the next step.

Synthesis of OCT(Phe)–PEG–NH_2

Octreotide was conjugated to Boc-NH-PEG-NHS using the following procedure. Briefly, Boc-NH-PEG-NHS, OCT, and EDC·HCl were dissolved in 5 mL acetonitrile (molar ratio of Boc-NH-PEG-NHS:OCT = 3:1) in the presence of 5 μL triethylamine. The mixture was stirred at 4°C for 12 h. Because OCT could be PEGylated at the N-terminus (phenylalanine, Phe) and the lysine (Lys) residue, the crude product was purified by loading directly onto a reversed-phase high-performance liquid chromatography (HPLC) system (LC-10AT; Shimadzu, Kyoto, Japan) with Delta-Pak C_{18} 25 × 100 mm^2 column and eluted with an acetonitrile gradient (35%–50%, v/v) in water [0.1% trifluoroacetic acid (TFA)] at a column temperature of 25°C. The flow rate was 1.0 mL/min, and UV absorbance was monitored at 280 nm. Then, the separated OCT(Phe)–PEG–NH–Boc was deprotected to obtain OCT(Phe)–PEG–NH_2 in the solvent of acetonitrile–TFA (80/20) solutions using an ice-water bath under argon.

Synthesis of OCT(Phe)–PEG–PTX and mPEG–PTX

Octreotide(Phe)–PEG–PTX and mPEG–PTX were synthesized by coupling the carboxyl group of 2′-SA-PTX with the amine group of OCT(Phe)–PEG–NH_2 and mPEG–NH_2, respectively, in the presence of EDC·HCl and NHS. Briefly, 24 mg 2′-SA-PTX was dissolved in 4 mL DMF. EDC·HCl (20 mg) and NHS (20 mg) were successively added to the above solutions. The mixture was stirred at room temperature for 2 h. Then, 120 mg OCT(Phe)–PEG–NH_2 or 100 mg mPEG–NH_2 dissolved in 2 mL DMF was added, and the mixture was stirred for another 24 h. Progress of the reaction was monitored by TLC [ethyl acetate:*n*-hexane = 6:1 (v/v)]. After the reaction was complete, DI water was added gradually, and the solution was dialyzed against DI water for 48 h (MWCO, 3.5 kDa) followed by lyophilization. The yield of OCT(Phe)–PEG–PTX and mPEG–PTX was 89.3% and 92.4% with respect to the PTX, respectively. The high yields could potentially assist in the industrialization of the conjugate manufacturing process. The resulting products were stored at 4°C until further use.

Characterization of 2′-SA-PTX, OCT(Phe)–PEG–NH_2, OCT(Phe)–PEG–PTX, and mPEG–PTX

Chemical structures of 2′-SA-PTX, OCT(Phe)–PEG–NH_2, OCT(Phe)–PEG–PTX, and mPEG–PTX were confirmed using a 500-MHz Avance Bruker NMR spectrometer, with OCT, mPEG, and PTX as controls. PTX, 2′-SA-PTX, OCT(Phe)–PEG–PTX, and mPEG–PTX were dissolved in $CDCl_3$, and OCT, mPEG, and OCT(Phe)–PEG–NH_2 were dissolved in D_2O.

Circular dichroism (CD) spectra of OCT, OCT(Phe)–PEG–NH_2, and OCT(Phe)–PEG–PTX were recorded in the range of 190–250 nm by using Jasco J-810 spectropolarimeter (Jasco, Easton, Maryland) with a CD cell of 0.1-cm path length and 1-nm bandwidth. Scan speed of 100 nm/min was used, and in all six scans were performed per sample. The spectra were expressed as mean residue molar ellipticity in deg·cm^2/dmol. Peptide concentrations were set at 100 μg/mL in 0.01 M phosphate buffer (pH 7.4).

Drug concentration in OCT(Phe)–PEG–PTX and mPEG–PTX prodrugs was determined using ultraviolet visible