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Peptide functionalized poly ethylene glycol-poly caprolactone nanomicelles for specific cabazitaxel delivery to metastatic breast cancer cells

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ABSTRACT

Metastatic cancer is responsible for 90% of deaths in world. Usage of nano-carriers improve the delivery and efficacy of chemotherapeutic agents. Recent studies suggest that decoration of the surface of nano-carriers with various targeting agents may further improve their overall therapeutic efficacy. Using specified peptides in targeted drug delivery is a key point in recent researches. In this study, tumor metastasis targeting (TMT) homing peptide was applied as a targeting group to improve specific drug delivery to tumor cells. TMT peptide is conjugated to poly ethylene glycol-poly caprolactone (PEG-PCL) micellar nanoparticles as carriers for targeted delivery of cabazitaxel to metastatic breast cancer cells. Synthesis of PEG-PCL copolymer was performed by amidation reaction between carboxylic acid group of PEG and amine group of PCL. Nanomicelles were prepared via solvent evaporation method. TMT peptide was covalently conjugated onto nanomicelles through the amine group of PEG. TMT-PEG-PCL nanoparticles were analyzed by Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), dynamic light scattering (DLS), gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR). Toxicity and cellular uptake of nanomicelles were investigated by in vitro cytotoxicity assays and confocal scanning microscopy in MCF-7 (non-metastatic breast cancer cells) and MDA-MB-231 (metastatic breast cancer cells). The final nanomicelles had about 110 nm mean size and encapsulation efficiency of 82.5%. Treatment of metastatic breast cancer cells with targeted nanomicelles significantly increased the necrosis rate to 65%, compared to 33% in non-targeted nanomicelles and 8% in control group. The MDA-MB-231 cells treated with targeted nanomicelles exhibited a strong increase in the fluorescence intensity of coumarin in comparison to the cells treated with non-targeted nanomicelles ($p < 0.001$). It could be concluded that the present carrier has the potential to be considered in treatment of metastatic breast cancer cells.

1. Introduction

Cabazitaxel (CBZ) is a semi-synthetic derivative of a natural toxoid, which is commercially applied for treatment of patients with hormone-refractory metastatic prostate cancer previously treated with a docetaxel-containing regimen. Previous studies showed that CBZ has antitumor activity against both docetaxel-sensitive and docetaxel-insensitive tumor models. A challenging drawback of CBZ is its poor water solubility, due to its bulky polycyclic structure. Tween 80 and ethanol has been used in CBZ formulation to increase its solubility which may cause severe side effects such as hypersensitivity and neurotoxicity [1,2]. An advantageous approach for overcoming this problem is the use of nanoparticles (NPs)

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containing encapsulated, dispersed, absorbed or conjugated drugs. NPs have unique characteristics which can lead to increase therapeutic index of chemotherapeutic drugs [3]. This outcome is mainly owed to the preferred localization of anti-cancer drugs in tumor by NPs. Nanocarriers such as liposomes and micelles show improvement in pharmaceutical properties of commercially available traditional formulations. NPs with hydrophilic surfaces can avoid opsonization and removal by the mononuclear phagocytic cells, stay away from extravasation through the continuous capillaries of healthy tissues and escape filtration by kidneys [4]. NPs with size between 50 and 150 nm can utilize the angiogenic vasculature of tumors for accumulation into tumor sites. Because of poor lymphatic drainage at tumor sites, results in a phenomenon known as the enhanced permeability and retention (EPR) effect [4]. Between different drug delivery systems, polymeric micelles are include of a hydrophilic shells which most of time is polyethylene glycol and a hydrophobic core that is reservoir for hydrophobic chemotherapeutic drugs such as CBZ [4,5]. Polymeric micelles (PMs) are spherical, nano-sized (10–200 nm) aggregates of amphiphilic copolymers that can be used as a delivery agent for breast cancer chemotherapy. PMs show different advantages over traditional drug delivery systems such as: Easy intravenous administration by increment of hydrophobic drugs solubility [6]. The hydrophilic micellar corona, in turn, forms a hydrating layer on the surface of the micelle that hinders plasma protein adsorption and subsequent rapid phagocytic clearance by the reticuloendothelial system (RES) [7]. PMs have notable potential for modifications, in which additional functional groups can be attached to the surface and used to modulate micelle properties. Particularly, the addition of a targeting ligand can increase the specificity of drug delivery to tumors [8]. They show low critical micelle concentrations (CMCs) attributing to small micellar size resulting in long-circulating and stable constructs [9].

Decorating the drug carrier with tumor cell can be facilitating the uptake of the drugs into the cells. This molecules will result in [1] higher retention of the drug carriers at tumor sites (i.e., by reducing passive transport away from tumor); and [2] improve uptake of the drugs by tumor cells.

Targeted delivery of the drugs is an approach for both increasing the efficiency and decreasing the side effects, especially for anticancer drug delivery [10]. Among different targeting strategies, antibodies are used as tumor-binding ligands, but a more fruitful approach is to utilize tumor specific peptides. The smaller size of peptides allows for better tumor penetration. In addition, the technology of peptide chemistry and engineering is more amenable for drug development. Tumor targeting with peptide ligands for cancer imaging and therapy has been extensively evaluated [11].

Peptide-mediated targeted therapy is achieved either through direct conjugation of an anti-cancer drug to peptide ligands or through preparation of peptide-guided drug carriers. Compared to peptide-drug conjugates, carriers bearing multiple tumor targeting peptides display increased affinity for cancer cells overexpressing the appropriate receptors, show better pharmacokinetics/tumor accumulation and are more likely to be internalized via receptor mediated endocytosis [12–15].

A cyclic ten-amino acid peptide (GCGNVVRQGC), referred to tumor metastasis targeting (TMT) peptide, is the ligand of XPNPEPZ, a subtype of aminopeptidase P. TMT peptide has been found to specifically bind to a series of highly metastatic tumor, such as PC-3M-1E8, MDA-MB-435S, MDA-MB-231, PG-BE1 and MKN-45sci, in vitro and in vivo, but not to the non-metastatic cell lines (such as PC-3M-2B4, MCF-7, PG-LH7 and NIH/3T3). This actually offers one potential avenue for developing nanocarriers that can specifically target and treat metastatic cancer [15,16].

As mentioned, CBZ is a commercial anticancer drug and is intensely attracted interests, due to outstanding properties such as high activity against both docetaxel-sensitive and docetaxel-insensitive tumor models [17]. This drug, but suffers the lack of solubility, which can be improved by loading on amphiphilic nanoparticles. Therefore, in this study we introduce PEG-PCL micelles for CBZ delivery. Moreover, TMT was used for more efficient targeting delivery. TMT efficaciously and selectively binds to metastatic tumor cells. In this study, we prepared a nanocarrier micelle system based on PEG-PCL. PEG-PCL micelles will improve the solubility problem of CBZ. TMT Homing peptide was conjugated to the surface of the NPs for promoting efficient and active targeting of the anticancer CBZ. In vitro delivery efficiency of the CBZ loaded TMT modified NPs was evaluated by comparing the cytotoxicity and cellular uptake of non-targeted and peptide-targeted formulations in MDA-MB-231 (metastatic breast cancer) cell line and MCF-7 (non-metastatic breast cancer) cells.

2. Materials and methods

2.1. Materials, cell lines

NH_2-PEG-COOH (Mw = 5000 Da), ε-caprolactone (ε-CL), stannousoctoate ($Sn(Oct)_2$) were purchased from Sigma-Aldrich Chemical Corp. (Shanghai, China); Cabazitaxel was supplied by the Shanghai Institute of Pharmaceutical Industry (Shanghai, China). Dulbecco's Modified Eagle Medium (DMEM) High Glucose was purchased from Hy Clone Thermo Scientific. Fetal Bovine Serum (FBS) was purchased from Gibco-Life Technologies. Penicillin/streptomycin and 0.25% (w/v) trypsin–0.1% (w/v) Ethylene Diamine Tetraacetic Acid (EDTA) were purchased from Solarbio (Beijing Solarbio Science and Technology, China). 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were purchased from sigma (USA). Culture flasks and dishes were from Corning (Corning, NY, USA). HPLC-grade acetonitrile was purchased from Anaqua Chemical Supply (Houston, TX, USA). Ethanol absolute, Tween 80, ammonium acetate of AR grade and spectrographic grade potassium bromide (KBr) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were of analytical reagent grade and purchased from commercial sources.

The metastatic breast cancer cell lineMDA-MB-231 and non-metastatic breast cancer cell line MCF-7 obtained from American Type Culture Collection (Manassas, VA) and cultured in RPMI-1640 and DMEM media respectively supplemented with 10% fetal bovine serum (FBS), penicillin (100 U mL^{-1}), streptomycin (100 μg mL^{-1}) at 37 °C in a humidified incubator supplemented with 5% carbon dioxide.

2.2. Preparation of nanocarrier, Boc-protection of amine group

Amino end of poly (ethylene glycol) 2-aminoethyl ether acetic acid (Mn = 5000D) and 3-aminopropan-1-ol were separately protected by di-tert-butyl dicarbonate (Boc_2O) according to the previously reported procedures. To a magnetically stirred mixture of amine (2 mmol) and $(Boc)_2O$ (2 mmol, 1 equiv) in a 25 mL round bottom flask, was added pyridine (80microlit) and the mixture was stirred at room temperature (30–35 °C) until completion of the reaction (24 h). The reaction mixture was extracted with Ethyl acetate (3 × 5 mL). The solvent was evaporated and the product was washed out with Et_2O. The Boced product was obtained as a solid (95%), characterized by NMR and FT-IR [18].

2.3. Polymerization of ε-caprolactone

N-protected 3-aminopropan-1-ol was used as initiator for ring opening polymerization of ε-caprolactone. Synthesis was carried out in a three-necked round bottomed flask (100 mL) equipped with a thermometer, a condenser and magnetic stirrer. The flask was purged with argon, evacuated twice and stored under an inert atmosphere. Argon was blown through the water absorption system with silica gel. A mixture of N-Boced 3-aminopropan-1-ol (0.9 cm^3) and $Sn(Oct)_2$ (90 μL) was added to toluene at 90 °C and stirred for 30 min. The ε-caprolactone (8.9 cm^3) monomer was added to the reaction mixture and stirring continued at 110 °C for 24 h. The reaction temperature was maintained using a silicone oil bath. The polymer was precipitated by the addition