Biomaterials 32 (2011) 9457–9469

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Self-aggregated pegylated poly (trimethylene carbonate) nanoparticles decorated with c(RGDyK) peptide for targeted paclitaxel delivery to integrin-rich tumors

Xinyi Jiang [a,b], Xianyi Sha [a,b], Hongliang Xin [a,b], Liangcen Chen [a,b], Xihui Gao [a,b], Xiao Wang [a,b], Kitki Law [a,b,c], Jijin Gu [a,b], Yanzuo Chen [a,b], Ye Jiang [a,b], Xiaoqing Ren [a,b], Qiuyue Ren [a,b], Xiaoling Fang [a,b,*]

[a] Department of Pharmaceutics, School of Pharmacy, Fudan University, 826 Zhangheng Rd., Shanghai 201203, People's Republic of China
[b] Key Laboratory of Smart Drug Delivery, Ministry of Education & PLA, 826 Zhangheng Rd., Shanghai 201203, People's Republic of China
[c] Department of Pharmacy, Huashan Hospital, Fudan University, Shanghai 200040, People's Republic of China

ARTICLE INFO

Article history:
Received 7 August 2011
Accepted 17 August 2011
Available online 10 September 2011

Keywords:
Nanoparticles
PEG-PTMC
Integrin protein
Cyclic RGD
Paclitaxel

ABSTRACT

Cyclic RGD peptide-decorated polymeric micellar-like nanoparticles (MNP) based on PEGylated poly (trimethylene carbonate) (PEG-PTMC) were prepared for active targeting to integrin-rich cancer cells. An amphiphilic diblock copolymer, α-carboxyl poly (ethylene glycol)-poly (trimethylene carbonate) (HOOC-PEG-PTMC) , was synthesized by ring-opening polymerization. The c(RGDyK) ligand, a cyclic RGD peptide that can bind to the integrin proteins predominantly expressed on the surface of tumor cells with high affinity and specificity, was conjugated to the NHS-Activated PEG terminus of the copolymer. The c(RGDyK)-functionalized PEG-PTMC micellar nanoparticles encapsulating PTX (c(RGDyK)-MNP/PTX) was fabricated by the emulsion/solvent evaporation technique and characterized in terms of morphology, size and zeta potential. Cellular uptake of c(RGDyK)-MNP/PTX was found to be higher than that of MNP/PTX due to the integrin protein-mediated endocytosis effect. In vitro cytotoxicity, cell apoptosis and cell cycle arrest studies also revealed that c(RGDyK)-MNP/PTX was more potent than those of MNP/PTX and Taxol. Pharmacokinetic study in rats demonstrated that the polymeric micellar nanoparticles significantly enhanced the bioavailability of PTX than Taxol. In vivo multispectral fluorescent imaging indicated that c(RGDyK)-MNP/PTX had high specificity and efficiency in tumor active targeting. Therefore, the results demonstrated that c(RGDyK)-decorated PEG-PTMC MNP developed in this study could be a potential vehicle for delivering hydrophobic chemotherapeutic agents to integrin-rich tumors.

1. Introduction

Over the last 5 decades, dozens of chemotherapeutic agents have been tested as single agents and in different combinations for the prevention and treatment of cancers. However, most current antineoplastic agents do not greatly differentiate between cancerous and normal cells, leading to systemic toxicity and adverse effects [1]. Advances in nanotechnology have shown promise in improving the therapeutic index of chemotherapeutic agents in cancer therapy [2–5]. In recent years, a variety of nano-containers and devices such as liposomes, polymeric nanoparticles or micelles, silica nanoparticles, and carbon nanotubes, have been designed to aid the transport of diagnostic or therapeutic agents to cancer cells [1,2,6–8]. Among these nanocarriers, self-assembled nanoparticles, composed of polymeric amphiphiles, have a potential to bring several advantages to therapeutic systems because of the high drug loading capacity, long circulation time, and controlled release profiles [9–12].

Poly (trimethylene carbonate) (PTMC), a biodegradable polyester, has been widely used in biomedical field due to their tunable biodegradability and biocompatibility [13]. PTMC homopolymer and its block copolymer with monomethoxy poly (ethylene glycol) (MPEG-PTMC) are stable in water, but could be degraded in vivo or in lipase solutions by an enzymatic surface erosion process without the formation acidic compounds [13–15]. Although high molecular weight PTMC is amorphous, PTMC with a relatively low molecular weight is semi-crystalline and has a melting temperature close to body temperature [16]. These unique properties render PTMC homo- and copolymers potential candidates for biomedical application such as controlled drug delivery. PEG as water soluble, biocompatible, non-toxic and nonimmunogenic material, could not only enhance biocompatibility but also favorably affect pharmacokinetics and tissue distribution [17–19].

* Corresponding author. Department of Pharmaceutics, School of Pharmacy, Fudan University, 826 Zhangheng Rd., Shanghai 201203, People's Republic of China. Tel.: +86 21 51980071; fax: +86 21 51980072.
E-mail address: xlfang@shmu.edu.cn (X. Fang).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.08.055

The integrin $\alpha_v\beta_3$, an important biomarker, is particularly known for its role in cancer progression and over-expressing in sprouting tumor vessels and most tumor cells [20]. The RGD (arginine-glycine-aspartic acid) short peptides can specifically bind with integrin $\alpha_v\beta_3$ and plays a significant role in regulating tumor growth, metastasis and tumor angiogenesis. The high affinity interaction between RGD peptides and cancer-related integrins has led to the widespread use of RGD peptide sequences as ligands for integrin-targeted drug and gene delivery applications [21–23].

In this study, an amphiphilic diblock copolymer, α-carboxyl poly (ethylene glycol)-poly (trimethylene carbonate) (HOOC-PEG-b-PTMC) was synthesized and a cyclic RGD peptide was conjugated to the PEG terminus of the copolymer. In an aqueous medium, these copolymers self-aggregate to form core–shell type nanoparticles. The hydrophobic core may serve as a nanoreservoir for loading hydrophobic drugs and the PEG shell could endow the nanoparticles with a lower level of the reticuloendothelial system (RES) uptake and hence a prolonged circulation half-life.

Paclitaxel (PTX), an anticancer drug approved by the FDA, has demonstrated significant anti-neoplasic activity including ovarian and breast cancer, non-small cell lung carcinoma, melanoma, head and neck cancer, and AIDS-related cancer [24]. However, its short circulation half-life, poor aqueous solubility, commonly occurring drug resistance [25,26] and serious side effects due to its non-selective distribution in vivo when delivered in conventional formulations usually compromise its clinical efficacy [27]. In this paper, c(RGDyK)-decorated PEG-PTMC diblock copolymer micellar-like nanoparticles, designated as c(RGDyK)-MNP, was used as the platform for targeted PTX delivery to integrin αvβ3-rich tumor. The system was carefully characterized, and the targeting efficiency was systematically evaluated in vitro at the cellular level by performing intracellular accumulation, sub-cellular distribution, in vitro cytotoxicity, cellular apoptosis and cell cycle assay in integrin protein-overpressed tumor cells, and in vivo in subcutaneous xenograft nude mice model.

2. Materials and methods

2.1. Materials

Methoxyl poly (ethylene glycol) (MPEG-OH, Mn is 3.0 kDa) and α-carboxyl poly (ethylene glycol) (HOOC-PEG, Mn is 3.5 kDa) were obtained from JenKem technology Co. LTD (Beijing, China). Polymer grade 1, 3-trimethylene carbonate, namely, 1,3-Dioxan-2-One (TMC) was purchased from Adamas Corporation. (Shanghai local agent, China). Stannous octoate (Sn(Oct)2, Aldrich) was distilled prior to use. Cyclic RGD peptide c(RGDyK) (Mw = 619.51) was synthesized by the GL Biochem Ltd. (Shanghai, China). PTX was purchased from Xi'an San jiang Bio-Engineering Co. Ltd. (Xi'an, China). Coumarin 6, 3-(4, N,N′-Dicyclohexyl carbodiimide (DCC), N-hydroxysuccinimide (NHS), Biotinyl-N-hydroxy-succinimide (NHS-Biotin), 10 nm streptavidin labeled colloidal gold, 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and Hoechst 33342 were purchased from Sigma (St Louis, MO, USA). 1, 1′-dioctadecyl-3, 3, 3′, 3′-tetramethylindotricarbocyanine Iodide (DiR) was purchased from Biotium (Invitrogen, USA). Recombinat Human Integrin $\alpha_v\beta_3$ was purchased from R&D SYSTERM (USA). Annexin V-FITC Apoptosis Detection kit, MitoTracker Red, Micro BCA Protein assay kit and Cell Cycle and Apoptosis analysis Kit were purchased from Beyotime® Biotechnology Co. Ltd (Nantong, China). Cellulose ester membranes (dialysis bag) with a molecular weight cut-off value (MWCO) of 3500 (Greenbird Inc. Shanghai, China) were used in dialysis experiments. Penicillin–streptomycin, DMEM, fetal bovine serum (FBS) and 0.25% (w/v) trypsin solution were purchased from Gibco BRL (Gaithersberg, MD, USA). Purified deionized water was prepared by the Milli-Q plus system (Millipore Co., Billerica, MA, USA). All other reagents and chemicals were of analytical grade and were used without further purification.

2.2. Cell line

Integrin protein-overexpressed U87MG cells were obtained from Shanghai Institute of Cell Biology. Culture plates and dishes were purchased from Corning Inc. (NY, USA). It was cultured in special Dulbecco's modified Eagle medium (DMEM , Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 100 IU/ml penicillin and 100 μg/ml streptomycin sulfate. All the cells were cultured in incubators maintained at 37 °C with 5% CO_2 under fully humidified conditions.

2.3. Animals

Female BALB/c nude mice (20 ± 2 g) and female Sprague–Dawley (SD) rats (200 ± 20 g), supplied by Department of Experimental Animals, Fudan University (Shanghai, China), were acclimated at 25 °C and 55% of humidity under natural light/dark conditions. All animal experiments were carried out in accordance with guidelines evaluated and approved by the ethics committee of the College of Pharmacy, Fudan University (Shanghai, China).

2.4. Synthesis of c(RGDyK)-decorated PEG-PTMC

2.4.1. Synthesis of HOOC-PEG-PTMC and MPEG-PTMC

MPEG-PTMC and HOOC-PEG-PTMC diblock copolymers were synthesized by ring-opening polymerization with modified condition compared to the described previously [28,29]. Briefly, calculated amounts of HOOC-PEG or MPEG and TMC were transferred into a thoroughly dried glass flask with a magnetic stirring bar. The vessel was dried under vacuum for 10 min and purged with nitrogen. Sn(Oct)2 in anhydrous toluene solution was charged into the vessel by syringe. The flask was degassed by several vacuum–purge cycles that also removed the solvent introduced in the catalyst solution. The flask was then sealed under reduced pressure. Copolymerization was carried out at 140 ± 1 °C for 2 days. The product was purified by precipitating a polymer solution in chloroform into an excess of hexane. The purified copolymers were dried in a vacuum oven at 40 °C for 24 h and then stored in a desiccator under vacuum at −20 °C. The copolymer composition was studied by ^{1}H NMR spectra in $CDCl_3$ on a Mercury Plus 400 MHz spectrometer (USA). FTIR spectra (Avatar 360ESP) were obtained from a neat film cast from the chloroform copolymer solution between KBr tablets.

2.4.2. Synthesis of NHS activated PEG-PTMC (NHS-PEG-PTMC)

HOOC-PEG-PTMC (0.5 g, 0.053 mmol), DCC, (0.0219 g, 0.106 mmol, 2 × excess), NHS, (0.0122 g, 0.106 mmol, 2 × excess) and 5 ml of dichloromethane were added to a round-bottom flask equipped a magnetic stirring bar, attached to a nitrogen line and a bubbler. The reaction was maintained for 24 h at room temperature. The reaction mixture was then filtered, concentrated under reduced pressure, and precipitated in cold diethyl ether and dried in vacuo at room temperature to constant weight. The yield was found to be 0.361 g. The synthetic scheme is shown in Fig. 1B, and the detailed assignment for the ^{1}H NMR spectrum has been provided in the Results section.

2.4.3. Synthesis of c(RGDyK)-decorated PEG-PTMC (c(RGDyK)-PEG-PTMC)

NHS-PEG-PTMC (50 mg, 0.0053 mmol) solved in 1 ml DMF was added to a solution of 6.3 mg of c(RGDyK) in 0.1M HEPES, adjusting to pH 8.4 with N-methyl morpholine. The reaction was maintained for 24 h at room temperature under moderate stirring. Following this period the resulting reaction mixture was dialyzed against deionized water using cellulose ester membrane with a molecular weight cut-off of 3500 for 48 h in order to remove the uncoupled peptide. The c(RGDyK))-conjugation efficiency was measured using the Micro BCA Protein assay kit. The final solution was lyophilized and stored at −20 °C until use.

2.5. Preparation of the nanoparticles

The c(RGDyK)-decorated MNP/PTX was prepared through the emulsion/solvent evaporation technique according to the procedure described elsewhere [30]. Namely , 35 mg of MPEG-PTMC, 5 mg of c(RGDyK)-PEG-PTMC and 2.3 mg of PTX in 1 ml dichloromethane (DCM) added into 5 ml of 0.6% sodium cholate aqueous solution were slowly poured into the solution and then sonicated at 200 W on ice using a probe sonicator (Xin zhi Biotechnology Co. Ltd., China). The emulsion formed was added drop-wise on 25 ml of sodium cholate 0.3% under rapid magnetic stirring. After that, dichloromethane was evaporated by rotary vacuum at 37 °C. The formed MNP suspension was centrifuged using an amicon centrifugal filter device (MWCO 3500 Da) and washed twice with deionized water in order to completely remove excess sodium cholate. After discarding the supernatant, MNP was resuspended in 1 ml of physiological saline and kept at 4 °C for further use.

The preparation of fluorescein-labeled c(RGDyK)-MNP was the same as that of PTX-loaded nanoparticles, except that 16 μl coumarin 6 or 80 μl DiR (1 mg/ml stock solution in dichloromethane) was additionally added to dichloromethane containing copolymers before emulsification. Then, the free coumarin 6 or Dir was removed via CL-4B column (Hanhong Chemica Co. LTD, China).

2.6. HPLC analysis

The concentration of PTX in samples was measured via HPLC conducted by using a Shimadzu HPLC system equipped with a reversed-phase column (Gemini 5 μm C18, 200 mm × 4.6 mm, Phenomenex, California, USA), an LC-10ATVP pump, an SPD- 10AVP UV detector (Shimadzu, Kyoto, Japan) and an HS2000 interface (Hangzhou Empire Science & Tech, Hangzhou, China) operated at 227 nm. The