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Preferential tumor accumulation and desirable interstitial penetration of poly(lactic-co-glycolic acid) nanoparticles with dual coating of chitosan oligosaccharide and polyethylene glycol-poly(D,L-lactic acid)

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ABSTRACT

Despite advances in polymeric nanoparticles (NPs) as effective delivery systems for anticancer drugs, rapid clearance from blood and poor penetration capacity in heterogeneous tumors still remain to be addressed. Here, a dual coating of poly (ethylene glycol)-poly (D,L-lactic acid) (PEG-PDLLA) and water-soluble chitosan oligosaccharide (CO) was used to develop PLGA-based NPs (PCPNPs) with colloidal stability for delivery of paclitaxel (PTX). The PCPNPs were prepared by a modified nanoprecipitation process and exhibited homogeneous size of 165.5 nm, and slight positive charge (+3.54 mV). The single PEG-PDLLA-coated PLGA NPs (PPNPs) with negative charge (−13.42 mV) were prepared as control. Human breast cancer MDA-MB-231 cell and mice MDA-MB-231 xenograft model were used for *in vitro* and *in vivo* evaluation. Compared to Taxol®, both PCPNPs and PPNPs increased the intracellular uptake and exerted stronger inhibitory effect on tumor cells *in vitro*, especially for PCPNPs. Particularly, due to the near neutral surface charge and shielding by the dual coating, the blank cationic NP presented low cytotoxicity. With the synergistic action of PEG-PDLLA and CO, PCPNPs not only strongly inhibited macrophage uptake and extended the blood circulation time, but also improved the selective accumulation and interstitial penetration capacity to/in tumor site. Consequently, a significantly enhanced antitumor efficacy was observed for the cationic PCPNPs. Our findings suggest that, the dual PEG-PDLLA/CO coating can effective improve the tumor accumulation and interstitial penetration of NPs and, therefore may have great potential for tumor treatment.

Statement of significance

Rapid clearance from blood and poor penetration capacity in heterogeneous tumors represent great challenge for polymeric nanoparticles (NPs) as effective delivery systems for anticancer drugs. This study provides a promising cationic nanoparticle (PCPNPs) with dual coating of chitosan oligosaccharide (CO) and PEG-PDLLA to address the above problem. The PCPNPs prepared with 165.5 nm and slight positive charge (+3.54 mV) showed an improved accumulation and interstitial penetration capacity to/in tumor site, and thus led to an enhanced antitumor efficacy. This is the first time to report the cooperative effect of PEG-PDLLA and CO on PLGA NPs in this field. This work can arouse broad interests among researchers in the fields of nanomedicine, nanotechnology, and drug delivery system.

1. Introduction

Biodegradable polymeric nanoparticles (NPs) have attracted immense interests as the effective delivery systems of anticancer drugs [1,2]. Special attention has been given to

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poly(D,L lactide-co-glycolide) (PLGA) and poly(lactic acid) (PLA) because of their excellent endocytosis efficiency, high loading capacity for a wide range of therapeutic agents, easy preparation process and tunable release rate [3–5]. However, limited blood circulation lifetime, undesirable tumor accumulation and poor interstitial penetration are still great challenges in this field [6,7].

It is known that the majority of the NPs *in vivo* can be cleared by the interception/metabolism of reticular endothelial system (RES) [8], or rapid opsonization and ensuing sequestration by cells of mononuclear phagocytic system (MPS) [9,10]. Nanoparticles in the range of 70–200 nm are considered as an optimal range for leveraging the enhanced permeability and retention (EPR) effect and minimizing clearance by MPS in blood circulation [11]. An alternative well-accepted method for camouflaging or masking NPs is to hinder the hydrophobic and electrostatic interactions between the plasma proteins and particle surface by adsorbing or grafting shielding groups [12]. Among them, PEG appears as an ideal candidate, and was extensively used, since it has been shown to successfully weaken the MPS uptake and lead to prolonged blood circulation time [13]. Meanwhile, due to the highly hydrophilicity feature, PEG is normally conjugated on hydrophobic macromolecules to form amphiphilic properties, which can effectively prevent PEG detaching from the surface of nanoparticles [14,15]. Furthermore, the molecular weight, chemical constitutions, and surface charge of the PEG exert significant effect on the physicochemical property and thus the blood circulation time of the PEGylation NPs [16].

Currently, penetration capacity of NPs in tumor tissue has aroused great attention [17–20]. Previous investigations indicated that the structural and functional abnormalities of the tumor vasculature and the dense interstitial matrix in tumor tissue often pose a barrier against delivering therapeutics to the core solid tumors, where the most aggressive tumor cells harbors [2,21]. To overcome the above physical barrier, several strategies have been developed ranging from pretreatment by matrix modifiers or angiogenesis inhibitors [18] to prepare enzyme-responsive size-changing nanoparticles [19]. Besides, cationic nanoparticle, due to strong cellular interaction and good cellular uptake, exhibits improved penetration capacity in heterogeneous tumors [2,22]. However, as positively-charged entities, cationic NPs often present dramatic adsorption capacity to opsonins or to other negatively charged serum proteins in the blood stream, and thus exert negative effect on tumor accumulation. Meanwhile, the strong electrostatic interaction with cell surface often induces non-specific cell uptake and thus leads to high cytotoxicity by the cell membrane damage. Although some researchers have tried to use pH sensitive polymeric vector to reverse surface charge inside the cancer tissue to provide the cationic characteristic for NPs [23,24], the design of these NPs is often complicated and the process is also hard to control *in vivo*. Therefore, novel cationic NPs with both high tumor accumulation and enhanced interstitial penetration capability are highly desired for delivery of anticancer drugs.

As a natural material, chitosan oligosaccharide (CO), a low molecular weight product from chitosan, exhibits great solubility in water, nearly complete body absorption ability and positive charge [25] and has been found to possess various biological activities including immune potentiating, antitumor, antihypertensive, and antimicrobial actions [26–28]. To our knowledge, the combination of chitosan and PEG have been proved to be suitable materials for long circulating [29], however, the study of low molecular chitosan oligosaccharide from chitosan and the dual coating of CO and PEG-PDLLA on PLGA nanoparticles have not yet been reported. The introduction of appropriate CO and PEG-PDLLA not only endowed the NPs with prolonged blood circulation and preferential tumor accumulation, but also with improved interaction with cell and dense interstitial matrix in tumor tissue. To achieve this goal, PLGA NPs coated by the PEG-PDLLA and CO (Mw, 5000 kDa) (PCPNPs) was prepared by a modified nanoprecipitation approach [30]. Paclitaxel (PTX), one of the best antineoplastic agents, is used as a model drug in this study. The commercially formulated paclitaxel in the mixture of cremophorEL (Cr-EL) and dehydrated alcohol (1:1, v/v) (Taxol®) and the PEG-PDLLA-coated PLGA nanoparticles (PPNPs) with similar size were used for comparison. Protein absorption, suspension stability and macrophage uptake of various NPs were tested. The cellular uptake and cytotoxicity were *in vitro* investigated with human breast cancer MDA-MB-231 cells, their pharmacokinetics (PK) study, antitumor efficacy, whole-body and intratumoral biodistributions were *in vivo* evaluated in MDA-MB-231-bearing mice.

2. Materials and methods

2.1. Materials

PLGA with 50:50 M ratio (Mw, 5 kDa, 15 kDa) and PEG-PDLLA (Mw, 2000–2000 Da) were purchased from Jinan Dai Gang biological technology Co. (Jinan, China). Paclitaxel and Cremophor EL formulation (Taxol®) were purchased from Knowshine Pharmachemicals Inc. (China) and Bristol-Myers Squibb (Italy), respectively. Chitosan oligosaccharide (CO, Mw 5000 Da) was purchased from Zhejiang Jinke Pharmaceutical Co., LTD, China. Coumarin 6, 4′, 6-diamidino- 2-phenylindole (DAPI), and MTT were all purchased from Sigma–Aldrich, China. DiR iodide (1, 1-dioctadecyl-3, 3, 3, 3-tetramethyl indotricarbocyanine iodide) was purchased from Caliper Life Sciences (Hopkinton, MA). All solvents were HPLC grade and used without further pretreatment.

2.2. Preparation of PTX-loaded PCPNPs

PTX-loaded PCPNPs were prepared by a modified nanoprecipitation method in ethanol–water system. PTX (2 mg) and the PLGA (20 mg) were first dissolved in acetone (5 mL) and then was poured into 10 mL of a water/ethanol solution (1:1, v/v) containing 0.2% (w/v) PEG-PDLLA and 0–0.2% CO under gentle magnetic stirring (100–150 rpm), obtaining a milky colloidal suspension. The organic solvent was then evaporated under high vacuum at 40 °C. Thereafter, the NPs were washed by distilled water using a Pellicon®XL Tangential Flow Filtration (Millipore, Billerica, MA) with a molecular weight cut-off of 10 kDa. Finally, the NPs were then freezing-dried in liquid nitrogen and lyophilized for storage at −80 °C for later use. For comparison, the PEG-PDLLA-coated PLGA nanoparticles (PPNPs) were also prepared by the similar process only in the absence of CO. The same procedure was used for the synthesis of fluorescent coumarin 6-loaded PCPNPs except that PTX was replaced by 0.05 wt% coumarin 6.

2.3. Characterization of the nanoparticles

The morphology, particle size, and zeta potential of NPs were characterized by transmission electron microscopy (TEM, JEOL JEM-200CX) and dynamic light scattering (DLS, PSS Z380). To calculate the percentage of CO coated on the surface of PCPNPs, elemental composition was detected by Elemental Analyser (Model: Vario ELIII, Elementar Co, Germany). PTX concentration was measured by High Performance Liquid Chromatography (HPLC) system with a UV detector (Agilent Technologies Inc, Cotati, CA). An ODS column (Phenomenex, 250 mm × 4.6 mm, 5 μm) was used for analysis and the column temperature was kept at 30 °C. The mobile phase was consisted of acetonitrile and water (55/45, v/v). Flow rate was 1.0 mL/min and the detection wavelength was set at