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PEGylated poly(trimethylene carbonate) nanoparticles loaded with paclitaxel for the treatment of advanced glioma: In vitro and in vivo evaluation

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ABSTRACT

The aim of this study was to investigate the antitumor effect of paclitaxel (PTX)-loaded poly(ethylene glycol)–poly(trimethylene carbonate) (MPEG–PTMC) nanoparticles (NP) against gioblastoma multiforme (GMB). PTX-loaded NP (NP/PTX) were prepared with synthesized MPEG–PTMC by the emulsion/solvent evaporation technique. In vitro physiochemical characterization of those NP/PTX showed satisfactory encapsulation efficiency and loading capacity and size distribution. Cytotoxicity assay revealed that encapsulation in nanoparticles did not compromise the antitumor efficacy of PTX against U87MG cells. Pharmacokinetic study in rats demonstrated that the polymer micellar nanoparticles significantly enhanced the bioavailability of PTX than Taxol. In intracranial xenograft tumor-bearing mice, the accumulation of nanoparticles in tumor tissues increased distinctly after 12 h post i.v. More importantly, in vivo anti-tumor effect exhibited the median survival time of NP/PTX treated mice (27 days) was significantly longer than those of mice treated with Taxol (24 days), physiological saline (21 days) and blank MPEG–PTMC NP (21 days). Therefore, our results suggested that PTX-loaded MPEG–PTMC nanoparticles significantly enhanced the anti-glioblastoma activity of PTX and may be a potential vehicle in the treatment of high-grade glioma.

1. Introduction

According to the WHO classification of cerebral glioma, glioma is divided into four grades: I, II, III, and IV glioma (Kleihues et al., 1993; Louis et al., 2007; Zülch, 1980). And the low-grade tumors usually evolved into a high-grade glioblastoma multiforme (GBM). GBM are highly angiogenic tumors and, consequently, these tumors harbor new and leaky blood vessels and lack of the effective lymphatic drainage system (Law et al., 2004; Lupo et al., 2005; Plate and Risau, 1995). It could cause edema, which is the consequence of a local disruption of the BBB by impaired capillary endothelial tight junctions (Groothuis et al., 1991; Leggett et al., 1998; Lu et al., 2008; Sameshima et al., 2000; Schneider et al., 2004; Wolburg et al., 2003). According to the pathological conditions of glioma in different grades, the different strategies should be taken to the design of the drug delivery system targeting glioma. When the glioma is still low-grade, the BBB is intact and the drug delivery system should be able to cross the BBB and further target to glioma (Huang et al., 2011). However, when it develops to advanced-grade, drug delivery system can accumulate in the glioma by the enhanced permeability and retention (EPR) effect.

PTX, a promising anticancer drug isolated from the bark of *Taxus brevifolia*, has been proven effective in the treatment of gliomas (Nikanjam et al., 2007; Regina et al., 2008). Despite the successful properties exhibited by this drug for several cancers, its utility in the clinic is hampered by severe limitations such as poor aqueous solubility and serious side effects due to its non-selective distribution in vivo. Clinically, the current formulation for PTX is made with Cremophor EL (polyethoxylated castor oil and ethanol, 50:50. Taxol), which should be diluted in saline prior to intravenous administration. Unfortunately, this formulation caused severe adverse allergic reactions due to histamine release and hypersensitivity reactions (Musacchio et al., 2008). Various drug delivery systems (DDS) have been developed to overcome these deficiencies, including micelles, nanoparticles and liposomes (Danhier et al., 2009; Xin et al., 2010; Zhan et al., 2010; Zhao et al., 2009). Among these DDS being applied to PTX, self-assembled nanoparticles, composed of polymer amphiphiles, have been considered to be effective carriers because they can stay unrecognized during blood circulation, reduce the adverse reactions and increase the therapeutic efficacy.

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Poly(trimethylene carbonate) (PTMC), a biodegradable polyester, has attracted much research interest due to their tunable biodegradability and biocompatibility (Rokicki, 2000; Zhang et al., 2006c). PTMC homopolymer and its block copolymer were stable in water, but could be degraded in vivo or in lipase solutions by an enzymatic surface erosion process without the formation of acidic compounds (Han et al., 2009; Pego et al., 2003; Rokicki, 2000; Watanabe et al., 2007; Zhang et al., 2006c). Although PTMC with high molecular weight is amorphous, PTMC with a relatively low molecular weight is semi-crystalline and has a melting temperature close to body temperature (Zhu et al., 1991). 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 (Mao et al., 2005; Veronese, 2001). In present work, low molecular amphiphilic MPEG–PTMC containing high percentage of PEG segment was synthesized by ring opening polymerization. In an aqueous medium, these copolymers self-assemble 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.

In this study, we aimed to investigate the delivery efficiency of those copolymer-based nanocarriers for insoluble anticancer drugs, such as PTX, in vitro and in vivo. To achieve this aim, PTX-loaded MPEG–PTMC nanoparticles by the emulsion/solvent evaporation technique. The physicochemical characteristics, in vitro drug release, in vitro cell uptake characteristics and in vivo harmacokinetics in rats were investigated, respectively. And also, biodistribution and intracranial tumor accumulation of fluorescein-labeled NP were evaluated by non-invasive and real-time NIR imaging systems. Furthermore, the in vitro antitumoral activity of NP/PTX against U87MG cells was assessed and in vivo antitumor pharmacological effect in intracranial tumor-bearing mice was evaluated.

2. Materials and methods

2.1. Materials, cells and animals

PTX was purchased from Xi'an San jiang Bio-Engineering Co. Ltd. (Xi'an, China). Taxol injection (Anzatax Injection Concentrate, 30 mg/5 ml) was produced by FH Faulding & Co. Ltd. trading as David Bull Lab (Melbourne, Australia). Cremophor EL was kindly supplied by BASF Ltd. (Shanghai, China). Methoxy poly(ethylene glycol) (MPEG-OH, Mn is 3.0 kDa) was 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 octate ($Sn(Oct)_2$, Aldrich) was distilled prior to use. 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) was purchased from Biotium (Invitrogen, USA). Coumarin 6, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), was purchased from Sigma (St. Louis, MO, USA). Cellulose ester membranes (dialysis bag) with a molecular weight cut off value (MWCO) of 3500 (Greenbird Inc., Shanghai, China) were used in dialysis experiments. Deionized (DI) water was produced by a Millipore water purification system (Millipore Corporation, USA). All the other solvents were analytical or chromatographic grade.

U87MG cells were obtained from Shanghai Institute of Cell Biology. It was cultured in special Dulbecco's modified Eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco). Balb/c nude mice (4–5 weeks old) of 20 ± 2 g body weight and female Sprague–Dawley (SD) rats (200 ± 20 g) obtained from Experimental Animal Center of Fudan University. All animal protocols were approved by the Fudan University Institutional Animal Care and Use Committee. Mice were housed under standard humane conditions and had access to food and water ad libitum.

2.2. Preparation of MPEG–PTMC NP

MPEG–PTMC block copolymer was synthesized by the ring-opening polymerization with modified condition compared to the described previously (Zhang et al., 2006b). The details of the synthesis procedures as well as the NMR characterizations of the resulting copolymers can be found in the online version of this article as Supplementary Materials.

MPEG–PTMC nanoparticles were prepared through the emulsion/solvent evaporation technique according to the procedure described elsewhere (Zhang et al., 2004). Namely, 40 mg of MPEG–PTMC and different amounts of PTX in 1 ml dichloromethane 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 30 ml of sodium cholate 0.3% under rapid magnetic stirring. After that, dichloromethane was evaporated by rotary vacuum at 40 °C. Nanoparticles were then centrifuged at 14,000 rpm at 4 °C for 45 min. After discarding the supernatant, nanoparticles were resuspended in 1 ml of physiological saline and kept at 4 °C for further use.

The preparation of fluorescein-labeled NP 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).

2.3. Characterization of the nanoparticles

2.3.1. Particle size, surface charge and morphology

Particle mean size, size distribution and zeta potential of the NP were determined by dynamic light scattering (DLS) using a Zeta Potential/Particle Sizer Nicomp^{TM} 380 ZLS (Pss. Nicomp^{TM}, Santa Barbara, USA). The analyses were performed with 5 mW He–Ne laser (632.8 nm) at a scattering angle of 90° at 25 °C. Each freshly prepared sample was diluted to the appropriate concentration using DI water to avoid multi-scattering phenomena and was placed into a quartz cuvette. The reported experimental result of each sample was expressed as a mean size ± SD for three separate experiments. The morphological examination of NP was observed by transmission electron microscope (TEM) (JEOL JMPEG-PTMC-1230, Japan) at an acceleration voltage of 200 kV after negative staining with phosphotungstic acid solution (2%, w/v).

2.3.2. Determination of PTX-loading content and encapsulation efficiency

To investigate the drug loading content (DLC%) and encapsulation efficiency (EE%), different amounts of PTX were co-dissolved with MPEG–PTMC in the preparation process of NP. The NP/PTX were diluted by acetonitrile and the concentration of PTX 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), a LC-10ATVP pump, a SPD-10AVP UV detector (Shimadzu, Kyoto, Japan) and a HS2000 interface (Hangzhou Empire Science & Tech, Hangzhou, China) operated at 227 nm. The mobile phase was a mixture of acetonitrile and water (60:40, v/v), the flow rate was 1.0 ml/min,