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Biodegradable PEG-poly(ω-pentadecalactone-*co*-*p*-dioxanone) nanoparticles for enhanced and sustained drug delivery to treat brain tumors

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ABSTRACT

Intracranial delivery of therapeutic agents is limited by penetration beyond the blood-brain barrier (BBB) and rapid metabolism of the drugs that are delivered. Convection-enhanced delivery (CED) of drug-loaded nanoparticles (NPs) provides for local administration, control of distribution, and sustained drug release. While some investigators have shown that repeated CED procedures are possible, longer periods of sustained release could eliminate the need for repeated infusions, which would enhance safety and translatability of the approach. Here, we demonstrate that nanoparticles formed from poly(ethylene glycol)-poly(ω-pentadecalactone-*co*-*p*-dioxanone) block copolymers [PEG-poly(PDL-*co*-DO)] are highly efficient nanocarriers that provide long-term release: small nanoparticles (less than 100 nm in diameter) continuously released a radiosensitizer (VE822) over a period of several weeks *in vitro*, provided widespread intracranial drug distribution during CED, and yielded significant drug retention within the brain for over 1 week. One advantage of PEG-poly(PDL-*co*-DO) nanoparticles is that hydrophobicity can be tuned by adjusting the ratio of hydrophobic PDL to hydrophilic DO monomers, thus making it possible to achieve a wide range of drug release rates and drug distribution profiles. When administered by CED to rats with intracranial RG2 tumors, and combined with a 5-day course of fractionated radiation therapy, VE822-loaded PEG-poly(PDL-*co*-DO) NPs significantly prolonged survival when compared to free VE822. Thus, PEG-poly(PDL-*co*-DO) NPs represent a new type of versatile nanocarrier system with potential for sustained intracranial delivery of therapeutic agents to treat brain tumors.

1. Introduction

Over the past decade, advances in cancer nanotechnology have allowed delivery of therapeutic agents to tumors with significantly decreased systemic toxicity [1–3]. When administered systemically, nanoparticle (NP)-based drug delivery systems can protect encapsulated therapeutic agents from rapid clearance through enzymatic digestion, renal filtration, or phagocytosis by the reticuloendothelial system [4,5]. Further, those nanoparticles with their surfaces modified by hydrophilic polymers such as poly(ethylene glycol) (PEG) or hyperbranched polyglycerols (HPG) have low tendency to form aggregates and reduced uptake by phagocytes, further prolonging their circulation times and retention at targeted sites [6–8]. Nevertheless, sustained release of encapsulated agents is difficult to achieve in most NP formulations: usually, the size of particles must be greater than 300–400 nm to be capable of releasing drug at controlled rates for more than one week [9–11]. But some applications require smaller particles; NPs with diameters smaller than 100 nm that provide low bursts and sustained release could enable new clinical applications.

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Previously, attempts to use nanomaterials for delivery of therapeutic agents into the brain have largely been stymied by the blood-brain barrier (BBB) [12,13]. Even particles modified to enhance penetration through the BBB have <1% of injected dose reaching the brain tissue after systemic administration [14–16]. But drugs can be administered directly to the brain by infusion in fluids. For example, in convection-enhanced delivery (CED), fluid is introduced into the brain via trans-cranial catheters; fluid infusion is controlled by a hydrostatic pressure gradient [17]. CED can be safely performed in humans [18], but one of the major challenges with CED is that most drugs disappear from the brain quickly after the end of the infusion period [19]. To extend the period of treatment after a CED infusion, suspensions of sustained release NPs have been infused into the brain [20,21]. CED of NPs to treat brain tumors is improved with NPs <100 nm in diameter, because of their enhanced penetration through the brain parenchyma compared to larger particles [21]. The introduction of PEG surface coatings may also enhance the distribution of NPs after CED [22], although size appears to be a more important factor than surface chemistry in determining the volume of distribution during NP CED [23].

Poly(ω-pentadecalactone-*co*-*p*-dioxanone) [poly(PDL-*co*-DO)] is a family of biocompatible and biodegradable copolyesters that can be formed into NPs [24]. Previous studies show that the copolymers degrade hydrolytically under physiological conditions at controlled rates over a period of several months due to their unique isodimorphic properties and random distribution of ω-pentadecalactone (PDL) and *p*-dioxanone (DO) repeat units in the polymer chains [25]. Modulation of the copolymer composition is an effective means to tune drug release rates from poly(PDL-*co*-DO) NPs [24]. Although the poly(PDL-*co*-DO) NPs are capable of delivering agents at sustained rates, formulations described thus far yield NPs with diameters of 200–400 nm, which produce small volumes of distribution when administered by CED [26].

Here, we tested the hypothesis that conjugation of PEG to poly(PDL-*co*-DO) chains would yield PEG-poly(PDL-*co*-DO) block copolymers that can be formulated into small nanoparticles (<100 nm) with two key properties for intracranial drug delivery: penetration to large volumes when administered by CED and release of drugs over long periods. We found that PEG-poly(PDL-*co*-DO) NPs release hydrophobic therapeutic agents over a period of more than 28 days *in vitro* and penetrate in the brain tissues of rats after CED. To show that these properties enhance the treatment of intracranial tumors, PEG-poly(PDL-*co*-DO) NPs encapsulating an inhibitor of the ataxia telangiectasia-related (ATR) protein and known radiosensitizer, VE822, were administered by CED to rats with intracranial tumors [27]. A single infusion of VE822 NPs improved survival of animals receiving fractionated radiotherapy. PEG-poly(PDL-*co*-DO) copolymers are new materials with potential to improve the delivery of potent agents for treating brain tumors.

2. Materials and methods

2.1. Materials

ω-Pentadecalactone (PDL), poly(ethylene glycol) methyl ether ($M_n = 2000$ Da, MeO-PEG2K–OH), and Novozym 435 catalyst (*Candida antarctica* lipase B or CALB supported on acrylic resin) were obtained from Sigma-Aldrich Chemical. The enzyme catalyst was dried at 40 °C under 2.0 mmHg overnight prior to use. *p*-Dioxanone (DO) was acquired from Leap Labchem Scientific Co. in China. Aceonitrile and dimethylsulfoxide were obtained from J.T. Baker (Avantor Performance Materials, Central Valley, PA, USA). VE822 was obtained from Selleck (Houston, TX, USA). DiI dye was purchased from Thermo Fisher Scientific (Waltham, MA, USA). RG2 cells were purchased from ATCC (Manassas, VA). Normal Human Astrocytes were obtained from Tim Chan at Memorial Sloan Kettering Cancer Center. All cells were cultured in 5% CO2 and air humidified in a 37 °C incubator. Each cell line was cultured up to passage number 20.

2.2. Enzymatic synthesis of PEG-poly(PDL-co-DO) diblock copolymers

The reaction substrates, ω-pentadecalactone (PDL), *p*-dioxanone (DO) and poly(ethylene glycol) methyl ether ($M_n = 2000$ Da, MeO-PEG2K–OH) in various ratios (Table 1), were blended with Novozym 435 catalyst (5 wt% vs total substrate) and toluene solvent (200 wt% vs total substrate). The resultant mixtures were stirred at 70 °C under atmospheric nitrogen gas for 26 h. At the end of the reactions, *n*-hexane was added to the product mixtures to cause precipitation of the formed copolymers. The crude polymers were washed with *n*-hexane twice and then dissolved in chloroform. After filtration to remove the catalyst particles, the polymer solutions were dropwise added to *n*-hexane to re-precipitate the PEG-poly(PDL-*co*-DO) copolymers. Finally, the block copolymers were washed with *n*-hexane and dried under high vacuum (<1.0 mmHg) at 40 °C overnight. The structure and composition of the polymers were analyzed by both proton and carbon-13 NMR spectroscopy using an Agilent 500 spectrometer, and their molecular weights and polydispersity values were measured by gel permeation chromatography (GPC) in chloroform using polystyrene standards. Non-PEGylated poly(PDL-*co*-DO) copolymer was produced by an identical method, but without the addition of the poly(ethylene glycol) methyl ether.

PEG-poly(PDL-*co*-DO): (a) ^{1}H NMR ($CDCl_3$) (ppm) - *PDL units*: 4.05–4.13 (m, $-CH_2-CH_2-(CH_2)_{10}-CH_2-CH_2-COO-$), 2.27–2.36 (m), 1.61 (m), 1.26 (br.,); *DO units*: 4.36/4.27 (br., $-CH_2-CH_2-O-CH_2-COO-$), 4.13–4.19 (m, $-CH_2-CH_2-O-CH_2-COO-$), 3.79 (br.); *EO units*: 3.65; plus a small peak at 3.38 due to terminal $-OCH_3$ groups. (b) Selected ^{13}C NMR absorptions ($CDCl_3$) (ppm): 174.0 (PDL-PDL*-PDL/DO-PDL*-PDL, $-CH_2-CH_2-(CH_2)_{10}-CH_2-CH_2-COO-$), 173.8 (PDL-PDL*-DO/DO-PDL*-DO, $-CH_2-CH_2-(CH_2)_{10}-CH_2-CH_2-COO-$), 170.2, 170.1, 170.0 (PDL-DO*-PDL, PDL-DO*-DO/DO-DO*-PDL, DO-DO*-DO, $-CH_2-CH_2-O-CH_2-COO-$), 70.55 ($-CH_2-CH_2-O-$), 69.6/69.2 ($-CH_2-CH_2-O-CH_2-COO-$), 68.5–68.2 ($-CH_2-CH_2-O-CH_2-COO-$), 65.1/64.4 ($-CH_2-CH_2-(CH_2)_{10}-CH_2-CH_2-COO-$), 63.7/63.2 ($-CH_2-CH_2-O-CH_2-COO-$), 34.4/34.2 ($-CH_2-CH_2-(CH_2)_{10}-CH_2-CH_2-COO-$), 24.9–29.6 ppm ($-CH_2-CH_2-(CH_2)_{10}-CH_2-CH_2-COO-$).

Table 1
Analysis of PEG-poly(PDL-*co*-DO) diblock copolymers.

Polymer	PDL/DO/PEG2K (feed molar ratio)	Polymer yield[a]	PDL/DO unit ratio (mol/mol)	PEG Content (wt%)	M_w (Da)[b]	M_w/M_n[b]
P56D44	40:60:4.2	71%	56:44	40%	12300	1.7
P65D35	50:50:4.6	72%	65:35	38%	13400	1.8
P71D29	60:40:5.0	77%	71:29	36%	15100	1.8

[a] Calculated based on total monomer substrate weight.
[b] Measured by GPC using polystyrene standards.