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Research paper

In vivo fate of unimers and micelles of a poly(ethylene glycol)-block-poly(caprolactone) copolymer in mice following intravenous administration

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Abstract

Methoxy poly(ethylene glycol)-*b*-poly(caprolactone) (MePEG-*b*-PCL) copolymers with varying PEG block lengths and a constant PCL block length were synthesized by cationic ring-opening polymerization and used to form nano-sized micelles. Due to their small size and superior *in vitro* stability, the $MePEG_{5000}$-*b*-PCL_{5000} micelles were selected for further *in vitro* characterization and an *in vivo* evaluation of their fate and stability following intravenous (i.v.) administration. Specifically, $_3$H-labelled $MePEG_{5000}$-*b*-PCL_{5000} micelles were i.v. administered to Balb/C mice at copolymer doses of 250, 2 and 0.2 mg/kg in order to examine the distribution kinetics of (1) copolymer assembled as thermodynamically stable micelles, (2) copolymer assembled as thermodynamically unstable micelles and (3) copolymer unimers, respectively. Overall, it was found that when the copolymer is assembled as thermodynamically stable micelles the material is effectively restricted to the plasma compartment. Interestingly, the copolymer was found to have a relatively long circulation half-life even when administered at a dose that would likely fall to concentrations below the CMC following distribution. Analysis of plasma samples from this group revealed that even 24 h post-administration a significant portion of the copolymer remained assembled as intact micelles. In this way, this study demonstrates that the hydrophobic and semi-crystalline nature of the PCL core imparts a high degree of kinetic stability to this micelle system.

Keywords: Block copolymer micelle; Poly(ethylene glycol)-block-poly(caprolactone); Drug delivery; *In vivo* fate; Kinetic stability; Pharmacokinetics; Biodistribution

1. Introduction

In aqueous media, amphiphilic block copolymers self-assemble to form micelles when the copolymer concentration is at or above the critical micelle concentration (CMC). At concentrations below the CMC the copolymer is present in solution as unimers [1]. Hydrophobic (e.g. paclitaxel) or amphiphilic (e.g. amphotericin B) drugs may be incorporated into the copolymer micelles as a means to formulate or deliver these drugs [2–6]. These formulations have been studied quite extensively with many reports on the *in vivo* fate of the drug following i.v. administration in micelles [3,6–11]. However, only a few studies have examined the *in vivo* fate of the copolymer micelles [6,12–16] and unimers [6,13,15]. Specifically, Burt et al. studied the *in vivo* fate of paclitaxel and MePEG-*b*-poly(D,L-lactide) (MePEG-*b*-PDLLA) copolymers in rats following i.v. administration of a MePEG-*b*-PDLLA micelle formulation of this drug [6]. The *in vivo* pharmacokinetics and biodistribution for micelles prepared from tyrosine (Tyr)-PEG-*b*-PDLLA and tyrosyl-glutamic acid (Tyr-Glu)-PEG-*b*-PDLLA copolymers were also investigated by Kataoka's group [13].

To this point, the effect of the administered copolymer dose on the *in vivo* behavior of micelles as well as the fate of copolymer unimers and micelles remains relatively

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unexplored. One of the only studies that has examined the influence of copolymer dose on the distribution kinetics of block copolymers was recently reported by Kabanov's group [15]. Their study evaluated the pharmacokinetics and biodistribution of the Pluronic® triblock copolymer P85 following administration of copolymer concentrations ranging from below to above the CMC of the material after the dilution that occurs upon administration [15].

Additional studies on the *in vivo* behavior and fate of other copolymer unimer and micelle systems are necessary as they will ensure full exploitation of block copolymer micelles as a viable drug formulation strategy. In this study, MePEG-*b*-PCL copolymers of varying PEG block lengths (i.e. $M_n = 20{,}000$, 10,000 and 5000 g/mol) and a constant PCL block length (i.e. $M_n = 5000$ g/mol) were synthesized, characterized and the physico-chemical properties of micelles formed from this series of copolymers were evaluated. The $MePEG_{5000}$-*b*-PCL_{5000} micelle system was selected for an *in vivo* evaluation of copolymer fate and stability following i.v. administration due to its small size and superior *in vitro* stability. Specifically, the pharmacokinetics and biodistribution profiles of the copolymer were evaluated in mice following i.v. administration of three distinct doses of the copolymer.

2. Materials and methods

2.1. Materials

MePEGs ($M_n = 5000$, $M_w/M_n = 1.06$; $M_n = 10{,}000$, $M_w/M_n = 1.10$ and $M_n = 20{,}000$, $M_w/M_n = 1.10$ as determined by size-exclusion chromatography (SEC)) from Sigma–Aldrich (Oakville, ON, Canada) were dried twice by azeodistillation of toluene. The monomer, ε-caprolactone (CL), dichloromethane, and toluene were dried using calcium hydride and distilled prior to use. The tritium (^3H) radiolabelled compound acetyl chloride (CH_3COCl in dichloromethane) was obtained from America RadioLabelled Chemicals Inc. (St. Louis, MO) and used without further purification. All other chemicals were obtained from Sigma–Aldrich (Oakville, ON, Canada) and used as received.

2.2. Synthesis of MePEG-b-PCL copolymers

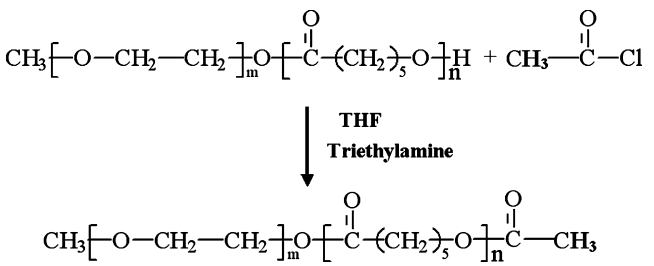
The block copolymer was synthesized using a metal-free cationic method [17]. A typical procedure for the synthesis of the copolymer was as follows: 1.0 g of MePEG (0.2 mmol, $M_n = 5000$, $M_w/M_n = 1.06$) was added to a flame-dried flask and dried twice by toluene azeodistillation. A 10 mL volume of dried dichloromethane and 1.0 g of CL (8.76 mmol, dried and distilled over calcium hydride) were then added to the flask. 0.6 mL (1 M, 0.6 mmol) of the catalyst and hydrogen chloride in diethyl ether was added to the mixture at 25 °C and the reactor was sealed using a rubber septum. The reaction mixture was maintained overnight with vigorous stirring at this temperature and terminated by the addition of 0.1 mL triethylamine. The precipitated triethylamine–HCl salt was removed by filtration and the copolymer was collected by precipitation in ether. The molecular weight of the PCL block was calculated from ^1H NMR and the known molecular weight of the MePEG precursor. ^1H NMR assignments were as follows: signals for the MePEG unit appeared at 3.38 ppm (3H, ***CH***$_3$–O–) and 3.60 ppm (4H, –O***CH***$_2$***CH***$_2$–); while, signals for the PCL block appeared at 1.35 ppm (2H, CO–CH_2–CH_2–***CH***$_2$–CH_2–CH_2–O), 1.55 ppm (4H, CO–CH_2–***CH***$_2$–CH_2–***CH***$_2$–CH_2–O), 2.28 ppm (2H, CO–***CH***$_2$–CH_2–CH_2–CH_2–CH_2–O), and 4.07 ppm (2H, CO–CH_2–CH_2–CH_2–CH_2–***CH***$_2$–O). The composition of the block copolymers was calculated by comparing the relative ratio of the PCL methylene proton signal at 4.07 ppm to the MePEG ethylene proton signal at 3.60 ppm [17].

2.3. Radiolabelling of MePEG-b-PCL copolymer

The radiolabelled block copolymer was synthesized by end-capping the terminus of the PCL block as shown in Scheme 1. Specifically, 10 mg of MePEG-*b*-PCL, that had previously been dried by toluene azeodistillation, was dissolved ($M_n = 10{,}000$ of 5000-*b*-5000 for MePEG-*b*-PCL, Table 1, entry 3) in 1.0 mL of dried THF and charged into a flame-dried glass tube reactor. The triethylamine in THF (1 mg) and 1 mCi of ^3H-labelled acetyl chloride were then added and left overnight at room temperature. The solvent was removed under vacuum. The triethylamine–HCl salt and unreacted acetyl chloride and its derivatives were removed by dialysis (Molecular weight cut-off, MWCO = 1000) against water (Spectrum Laboratories Inc., Dominguez, CA) and the remaining copolymer was dried by lyophilization.

2.4. Characterization of MePEG-b-PCL copolymers

^1H NMR spectra were obtained using a Mercury 400 spectrometer (400 MHz for ^1H, MR Resources Inc., Fitchburg, MA) with $CDCl_3$ as solvent. Chemical shifts were reported in ppm with $CHCl_3$ as the internal standard. SEC measurements were carried out at room temperature using a Waters 590 liquid chromatography system equipped with three Waters Styragel HR 4E columns and a 410 differ-



Scheme 1. The ^3H-labelled $MePEG_{5000}$-*b*-PCL_{5000} copolymer was prepared using an end-capping method. The three tritium atoms in the methyl group are shown in bold.