Biodistribution and Excretion of Radiolabeled 40 kDa Polyethylene Glycol Following Intravenous Administration in Mice

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ABSTRACT: The pharmacokinetics, excretion, and tissue distribution of [^{14}C]-labeled polyethylene glycol–alanine (PEG–Ala) were determined after slow bolus administration into the femoral vein of male CD-1 mice. The pharmacokinetics of PEG–Ala in blood and plasma revealed a biphasic elimination with a terminal half-life of 20 h. Eighty-five percent of the excreted material was voided in the urine and the remaining amount was detected in the feces. PEG–Ala-derived radioactivity was widely distributed with detectable levels of radioactivity observed in all tissues examined. The highest concentration was observed in the kidneys followed by lungs, heart, and liver. Six hours after administration, PEG–Ala levels were significantly reduced in all tissues. Despite a slow prolonged decrease, radioactivity was still detectable after 28 days. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2362–2370, 2013

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INTRODUCTION

Polyethylene glycol (PEG) is a unique polyether diol with a general formula of HO-(CH_2CH_2O)$_n$H. Pegylation is a proven drug delivery technology, and PEG is widely used in many marketed drugs. PEG is an amphiphilic polymer and highly hydophilic in aqueous solutions, resulting in an increase in hydrodynamic radius. The hydrodynamic radius of 40 kDa PEG exhibits a predicted molecular weight (MW) nearly 10-fold greater than the molecular mass as determined by MALDI-TOF-MS (matrix assisted laser desorption-ionization time of flight mass spectrometer) producing a surrounding water shell.[1] This increase in hydrodynamic radius may result in reduced renal clearance (CL) and a substantially prolonged residence time in the circulation leading to increased exposure to the molecule. This increased exposure thereby alters its pharmacokinetic as well as absorption, distribution, metabolism, and excretion *in vivo* characteristics. The stabilization of the water shell surrounding PEG conjugated to drug molecules protects the molecule from unfolding or adsorbing to surfaces, thereby enhancing the macromolecule's physical stability.[2] Additionally, DeNardo et al.[3] reported that because of steric hindrance, PEG protects macromolecules from proteolytic or nuclease activity, thus providing enhanced metabolic stability.

In addition, PEG has the ability to reduce biological immunogenicity by shielding antigenic epitopes through steric hindrance, making it an ideal molecule to conjugate to proteins[4,5] and small molecules.[6] Furthermore, *in vivo* studies have demonstrated that PEGylated compounds accumulate in tumors because of their leaky vasculature and reduced lymph drainage. This passive targeting mechanism has been described as the "enhanced permeability and retention" effect.[7,8] PEG is considered to be a molecule with unique physicochemical characteristics, and it has been reported to be safe in many cosmetic and food products.[9] US Food and Drug Administration approved PEG for human intravenous(ly) (i.v.), oral, and dermal applications. PEG is currently used in both experimental and marketed pharmaceutical products.[6]

To date, few investigations have been reported on the tissue distribution of PEG in spite of its extensive

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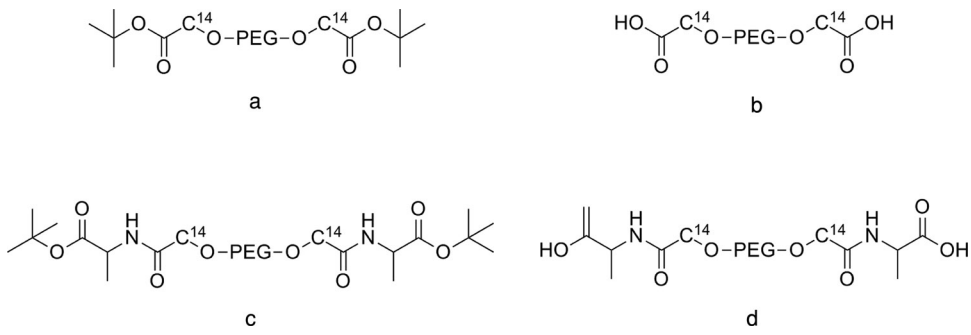

Figure 1. 40 kDa[^{14}C]PEG–Ala-di-acid and its precursors. (a) 40 kDa[^{14}C]PEG-di-ester, (b) 40 kDa[^{14}C]PEG-di-acid, (c) 40 kDa[^{14}C]PEG–Ala-di–*t*-butyl ester, and (d) 40 kDa[^{14}C]PEG–Ala-di-acid.

use as a polymeric modifier of protein drugs. The present study is designed to explore the biodistribution and excretion of radiolabeled PEG, 40,000 Da, with alanine attached to both ends of the polymer.

MATERIALS AND METHODS

Materials

[^{14}C]–PEG–alanine (PEG–Ala) of 40 kDa was synthesized with ^{14}C incorporated into the permanent ether bond backbone of PEG and purified to greater than 98% radiopurity at Chemsyn Laboratories (Lenexa, Kansas). PEG polydispersity was 1.05. Synthesis was performed according to published procedures[10] using [^{14}C]*t*-butyl bromoacetate. The specific activity of PEG–Ala was 0.336 mCi/g. PEG–Ala was chosen because it is the same PEG linker and spacer used in the clinical anticancer drug candidate Pegamotecan™ (PEG–Ala–camptothecin).

Animals

Studies were conducted in male CD-1 mice (Sprague–Dawley; Harlan, Indianapolis, Indiana) with an initial mean weight of 35 g. This study was conducted in accordance with the current guidelines for animal welfare and is in compliance with the NRC *Guide for the Care and Use of Laboratory Animals*[11] and the US Department of Agriculture Laboratory Animal Welfare Act.[12] Approvals for the following animal study were obtained from the Ethical Committees of Oread Biosafety, Inc. (Lawrence, Kansas).

Methods

General Chemical Procedures

Isotope [^{14}C] was incorporated into the backbone of PEG through a permanent ether bond (Fig. 1). The permanent ether bond is the chemical bond present in the backbone of PEG linking the repeating ethylene glycol units together. Hence, the retained isotope [^{14}C] implies that the detected radioactivity represents the presence of full-length PEG.

All reactions were conducted under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were dried under vacuum or by azeotropic distillation from toluene prior to use. NMR spectra were obtained using a Varian Mercury®300 NMR spectrometer (Palo Alto, CA) and deuterated chloroform solvent unless otherwise specified. Chemical shifts (δ) were reported in parts per million (ppm) downfield from tetramethylsilane.

Preparation of 40 kDa[^{14}C]PEG-Di-Ester

A solution of 40 kDa PEG-diol (5.0 g, 0.125 mmol) in toluene (65 mL) was azeotroped for 2 h with the removal of 10 mL of distillate (Fig. 1a). The reaction mixture was then cooled to 35°C, followed by the addition of 0.5 mL (0.5 mmol) of a 1.0 M solution potassium *t*-butoxide in *t*-butanol. The resulting mixture was stirred for 2 h at 35°C–40°C, followed by the addition of 0.20 g (1.0 mmol) of [^{14}C]*t*-butyl bromoacetate. The resulting cloudy mixture was stirred for 18 h at 35°C–40°C and then filtered through celite. The solvent was removed under reduced pressure. The residue was crystallized from chilled methylene chloride (DCM)/ethyl ether (100 mL, 1:4, v/v) to generate PEG di-ester (5 g). ^{13}C NMR (75.4 MHz; $CDCl_3$) δ 168.6, 80.75, 71.5 to 68.6 (PEG), 27.2.

Preparation of 40 kDa[^{14}C]PEG-Di-Acid

A solution of the above-mentioned PEG di-ester (5 g, 0.125 mmol) in trifluroacetic acid (TFA) and DCM (37.5 mL, 1:2, v/v) was stirred at room temperature for 2 h (Fig. 1b). The solvent was removed under reduced pressure, and the residue was crystallized from chilled DCM/ethyl ether (100 mL, 1:4, v/v). The wet solid isolated was redissolved in 0.5% $NaHCO_3$ (10 mL), followed by the extraction with DCM (50 mL). The organic solution was dried ($MgSO_4$), filtered, and solvent removed under reduced pressure. The residue was crystallized from chilled DCM/ethyl ether (100 mL, 1:4, v/v) to give PEG di-acid (4.3 g). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 170.3, 71.5 to 68.6 (PEG).