Journal of Controlled Release 226 (2016) 115–123

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Feasibility of poly(ethylene glycol) derivatives as diagnostic drug carriers for tumor imaging

Kengo Kanazaki [a,b], Kohei Sano [a,c], Akira Makino [a,d], Fumio Yamauchi [b], Atsushi Takahashi [b], Tsutomu Homma [b], Masahiro Ono [a], Hideo Saji [a,*]

[a] Department of Patho-Functional Bioanalysis Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto, 606-8501, Japan
[b] Medical Imaging Project, Corporate R&D Headquarters, Canon Inc., 3-30-2 Shimomaruko, Ohta-ku, Tokyo, 146-8501, Japan
[c] Kyoto University Hospital, 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan
[d] Biomedical Imaging Research Center, University of Fukui, 23-3 Matsuokashimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui, 910-1193, Japan

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 26 January 2016
Accepted 6 February 2016
Available online 8 February 2016

Keywords:
Cancer diagnosis
Poly(ethylene glycol)
Indocyanine green
Photoacoustic imaging
Single-photon emission computed tomography

ABSTRACT

Poly(ethylene glycol) (PEG) is an artificial but biocompatible hydrophilic polymer that has been widely used in clinical products. To evaluate the feasibility of using PEG derivative itself as a tumor imaging carrier *via* an enhanced permeability and retention (EPR) effect, we prepared indium-111-labeled PEG (^{111}In-DTPA-PEG) and indocyanine green (ICG)-labeled PEG (ICG-PEG) with PEG molecular weights of 5–40 kDa and investigated their *in vivo* biodistribution in colon26 tumor-bearing mice. Thereafter, single-photon emission computed tomography (SPECT) and photoacoustic (PA) imaging studies were performed. The *in vivo* biodistribution studies demonstrated increased tumor uptake and a prolongation of circulation half-life as the molecular weight of PEG increased. Although the observed differences in *in vivo* biodistribution were dependent on the labeling method (^{111}In or ICG), the tumor-to-normal tissue ratios were comparable. Because PEG-based probes with a molecular weight of 20 kDa (PEG20) showed a preferable biodistribution (highest accumulation among tissues excised and relatively high tumor-to-blood ratios), an imaging study using ^{111}In-DTPA-PEG20 and ICG-PEG20 was performed. Colon26 tumors inoculated in the right shoulder were clearly visualized by SPECT 24 h after administration. Furthermore, PA imaging using ICG-PEG20 also detected tumor regions, and the detected PA signals increased in proportion with the injected dose. These results suggest that PEG derivatives (20 kDa) serve as robust diagnostic drug carriers for tumor imaging.

1. Introduction

Nanosized delivery vehicles, including liposomes, nanomicelles, and nanoparticles, have a potential utility as diagnostic and therapeutic drug carriers targeting tumors because of their capacity to deliver a large drug payload [1,2]. In general, nanomaterials are rapidly endocytosed by the reticuloendothelial cells in the liver and spleen when administered intravenously [3], which could shorten the duration of *in vivo* circulation. This uptake decreases tumor uptake *via* the enhanced permeability and retention (EPR) effects by which macromolecules can preferentially accumulate within the tumor tissues [4,5]. To improve the *in vivo* pharmacokinetics and assure nanomaterial *in vivo* stability, most nanomaterials are coated with poly(ethylene glycol) (PEG) on their surface to increase particle hydrophilicity [6,7]. In addition, several bioactive proteins conjugated with biocompatible PEG have been widely used in clinical products [8,9] to optimize their *in vivo* biodistribution.

PEG characteristics include the following: 1) high solubility in aqueous solutions; 2) low toxicity and high biocompatibility [9]; 3) facile PEG modification through the terminal attachment of various types of functional groups; and 4) availability of a wide range of molecular weights [10]. Although high molecular weight PEG molecule itself could be speculated to exhibit increased tumor uptake *via* the EPR effect, there has been no report on this research. Therefore, we proposed that PEG derivatives (PEG chain possessing functional group at termini) can be novel diagnostic drug carriers for tumor imaging based on the EPR effect, and clarified favorable molecular weight of PEG for *in vivo* tumor imaging *via* EPR effect. In this study, we focused on two distinct imaging modalities: single-photon emission computed tomography (SPECT) and photoacoustic (PA) imaging. SPECT is a nuclear medical imaging technique widely used in clinical that enables the performance of a whole body scan. PA imaging is a form of optical imaging that has a potential for intraoperative diagnosis [11,12]. PA imaging noninvasively detects ultrasonic waves thermoelastically induced by optical absorbers (*i.e.*, a fluorescent dye and metal nanoparticles) irradiated with a pulsed laser [13]. The ultrasonic waves display much lower tissue scattering, leading to penetration depths of multiple centimeters and sub-

* Corresponding author.
E-mail address: hsaji@pharm.kyoto-u.ac.jp (H. Saji).

http://dx.doi.org/10.1016/j.jconrel.2016.02.017
0168-3659/

millimeter spatial resolution [14]. Thus, PA imaging has the potential for a broader clinical application than other forms of optical imaging, and the development of contrast agents for PA tumor imaging has been strongly desired. For SPECT and PA imaging, we prepared PEG molecules labeled with a radioisotope [indium-111: ^{111}In, $t_{1/2}$ = 2.8 days; γ-radiation, 171 keV (90%), 254 keV (94%)] using diethylene triamine pentaacetic acid (DTPA) as a metal chelator and PEG conjugated with a fluorescent dye (indocyanine green; ICG), respectively. ICG is a US Food and Drug Administration (FDA)-approved material that has been applied to the assessment of liver function and retinal angiography [15,16]. Because of absorptions in the near-infrared window [17], ICG is suitable for *in vivo* PA imaging [18–20].

Herein, this study first assessed the quantitative *in vivo* biodistribution of ^{111}In-DTPA-PEG and ICG-PEG in tumor-bearing mice because there was a possibility that pharmacokinetics of PEG derivatives could be altered relative to the physicochemical properties of the molecules (signal emitters) conjugated to PEG. Subsequently, we evaluated the feasibility of using PEG derivatives as tumor diagnostic drug carriers through an *in vivo* imaging study incorporating SPECT and PA imaging.

2. Material and methods

2.1. Materials

α-Aminoethyl-ω-methoxy poly(oxyethylene) (monoamino PEG, SUNBRIGHT ME-EA series, molecular weight: 5, 10, 20, and 40 kDa) and α-aminopropyl-ω-aminopropyl poly(oxyethylene) (diamino PEG, SUNBRIGHT DE-PA series, molecular weight: 20 kDa) were purchased from NOF Co. (Tokyo, Japan). Each monoamino PEG is hereinafter referred to as PEG5, PEG10, PEG20, and PEG40 according to the molecular weight of each PEG formulation. Hydrodynamic diameter of each monoamino PEG was measured by dynamic light scattering (DLS) with the Zetasizer nano (Malvern Instruments Ltd. (Worcestershire, UK)). S-2-(4-Isothiocyanatobenzyl)-diethylene triamine pentaacetic acid (*p*-SCN-Bn-DTPA) was purchased from Macrocyclics Inc. (Dallas, TX, USA). *N,N*-Diisopropylethylamine (DIPEA), potassium iodide, and iodine were purchased from Nacalai tesque, Inc. (Kyoto, Japan). Barium chloride was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). ICG was purchased from the Pharmaceutical and Medical Device Regulatory Science Society of Japan (Tokyo, Japan). 2-[7-[1,3-Dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2H-benzo[e]indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-[5-(3-sulfosuccinimidyl)oxycarbonylpentyl]-1H-benzo[e]indolium, inner salt, sodium salt (ICG-Sulfo-OSu) was purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). ^{111}In chloride ($^{111}InCl_3$) (74 MBq/mL in 0.02 N HCl) was purchased from Nihon Medi-Physics (Tokyo, Japan). All other chemicals used were of the highest purity available.

2.2. Preparation of PEG conjugates

2.2.1. DTPA-PEG

For radiolabeling with ^{111}In, a DTPA derivative cationic metal chelator was introduced to PEG. Each monoamino PEG (1.0 μmol in 1 mL chloroform) was mixed with *p*-SCN-Bn-DTPA [2.0 μmol in 1 mL *N,N*-dimethylformamide (DMF)] and DIPEA (2.0 μmol in 10 μL chloroform) at a molar ratio of PEG:DTPA = 1:2 and was incubated at room temperature (r.t.) for 24 h. After solvent evaporation, the resulting mixture was dissolved in Tris–HCl buffer (20 mM, pH 9.0) and purified by ultrafiltration with Amicon Ultra centrifugal filter units [molecular weight cut-off (MWCO): 3 kDa for PEG5 and PEG10 and 10 kDa for PEG20 and PEG40] (Merck Millipore, Co., Billerica, MA, USA) to remove unconjugated DTPA. After three times of ultrafiltration, the resulting solution was purified on an anion exchange chromatography system (AKTApurifier 10, GE Healthcare, Little Chalfont, UK) equipped with a Resource® Q 1 ml column (GE Healthcare) at a flow rate of 4 mL/min to obtain PEG conjugated with DTPA (DTPA-PEG). Tris–HCl buffer (20 mM, pH 9.0) was used as a running buffer, and DTPA-PEG was eluted using an NaCl concentration gradient (linear gradient of 0–1 M NaCl, 0–16 min). The elution of DTPA-PEG was confirmed by both PEG quantification and measurement of its absorbance at 254 nm for the quantitation of *p*-SCN-Bn-DTPA. The PEG concentration was determined as previously reported [21,22]. In brief, a PEG standard sample (1–20 μg/mL, 200 μL) or DTPA-PEG (200 μL) was mixed with 5% barium chloride in 1 M HCl aqueous solution (100 μL) and iodine aqueous solution [1.66% potassium iodide and 1.27% iodine in distilled deionized water (5 μL)] in a 96-well plate for 5 min, and their absorbance values at 535 nm were measured with an Infinite® 200 PRO plate reader (Tecan Japan Co., Ltd., Kanagawa, Japan). The PEG concentration was calculated from a standard curve obtained by plotting the absorbance value against the already-known PEG concentration. Each DTPA-PEG is hereinafter referred to as DTPA-PEG5, DTPA-PEG10, DTPA-PEG20, and DTPA-PEG40 according to the molecular weight of PEG.

For radiolabeling, the purified DTPA-PEG preparations were added to ^{111}In chloride (3.7 MBq) in 0.1 M sodium acetate (pH 6.0) and were incubated for 30 min. The radiochemical purity was analyzed by sodium dodecyl sulfate-poly(acrylamide) gel electrophoresis (SDS-PAGE, Novex® Tris-Glycine 4%–20% gel, Life Technologies Co.). Gel autoradiographic images were captured with the bioimaging analyzer BAS-5000 (Fujifilm Co., Tokyo, Japan). The radioactivity in the fractions of ^{111}In-DTPA-PEG and unbound ^{111}In were quantified using Multi Gauge V3.0 software (Fujifilm Co.). The gel was soaked in ddH_2O (100 mL) mixed with 5% barium chloride in a 1 M HCl aqueous solution (50 mL) and an iodine aqueous solution (1.66% potassium iodide and 1.27% iodine in ddH_2O, 2.5 mL) [21,22] and then incubated at r.t. for 5–10 min to develop PEG staining.

2.2.2. ICG-PEG

PEG conjugated with ICG was prepared as fluorescence and PA imaging probes. Each mono-amino PEG (0.54 μmol in 1 mL chloroform) was mixed with ICG-Sulfo-OSu [1.08 μmol in 100 μL dimethyl sulfoxide (DMSO)] at a molar ratio of PEG:ICG = 1:2, followed by incubation at r.t. for 24 h with light shielding. After solvent evaporation, the resulting mixture was dissolved in methanol (2 mL) and dialyzed against methanol with pre-treated regenerated cellulose (RC) membrane Spectra/Por® 7 dialysis tubing (MWCO: 3.5 kDa) (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) to remove unconjugated ICG. PEG quantification was performed as described above. To confirm the number of ICG molecules conjugated to PEG, the concentration of ICG in each ICG-PEG sample was calculated based on its absorption at 795 nm in the presence of 5% SDS using a UV–Vis–NIR system (UV-1800, Shimadzu Co., Kyoto, Japan). Each ICG-PEG is hereinafter referred to as ICG-PEG5, ICG-PEG10, ICG-PEG20, and ICG-PEG40 according to the molecular weight of PEG.

To determine chemical purity, purified ICG-PEG (100 pmol ICG) was subjected to SDS-PAGE separation. The gel was imaged with an IVIS imaging System 200 (Perkin Elmer Inc., ex/em 745/840 nm, exposure time: 1 s), and the fractions of covalently bound ICG to PEG were assessed by fluorescence intensity as previously reported [19]. Subsequently, PEG staining was performed as described above.

2.2.3. DTPA-PEG-ICG

Diamino PEG (molecular weight: 20 kDa) (1.08 μmol in 1 mL chloroform) was mixed with ICG-Sulfo-OSu (1.08 μmol in 100 μL DMSO) at a ratio of PEG:ICG = 1:1 and was incubated at r.t. for 24 h with light shielding. After solvent evaporation, the sample was dissolved in methanol (2 mL). The resulting solutions were dialyzed against methanol to remove unconjugated ICG-Sulfo-OSu. Subsequently, the solvent was evaporated, MES buffer (20 mM, pH 4.0) was added, and samples were purified using a cation exchange chromatography system equipped with a Resource® S 1 ml column (GE Healthcare) at a flow rate of 4 mL/min to remove unreacted PEG. MES buffer (20 mM,