Nuclear Medicine and Biology 42 (2015) 65–70

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

Integrity of ^{111}In-radiolabeled superparamagnetic iron oxide nanoparticles in the mouse☆

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ARTICLE INFO

Article history:
Received 31 July 2014
Accepted 29 August 2014

Keywords:
Radioactivity
Mouse
SPION

ABSTRACT

Introduction: Iron-oxide nanoparticles can act as contrast agents in magnetic resonance imaging (MRI), while radiolabeling the same platform with nuclear medicine isotopes allows imaging with positron emission tomography (PET) or single-photon emission computed tomography (SPECT), modalities that offer better quantification. For successful translation of these multifunctional imaging platforms to clinical use, it is imperative to evaluate the degree to which the association between radioactive label and iron oxide core remains intact in vivo.
Methods: We prepared iron oxide nanoparticles stabilized by oleic acid and phospholipids which were further radiolabeled with ^{59}Fe, ^{14}C-oleic acid, and ^{111}In.
Results: Mouse biodistributions showed ^{111}In preferentially localized in reticuloendothelial organs, liver, spleen and bone. However, there were greater levels of ^{59}Fe than ^{111}In in liver and spleen, but lower levels of ^{14}C.
Conclusions: While there is some degree of dissociation between the ^{111}In labeled component of the nanoparticle and the iron oxide core, there is extensive dissociation of the oleic acid component.

1. Introduction

Superparamagnetic iron oxide (SPIO) nanoparticles, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) with a particle size of 5–25 nm and appropriate surface coatings have potential as drug delivery nanoparticles because they can be targeted to a tumor or diseased organ by using an external magnet [1–3]. They also have high capabilities as magnetic resonance imaging (MRI) contrast enhancers [4,5]. The essential requirement for the use of SPIO nanoparticles in these applications is a narrow distribution of particle sizes smaller than 100 nm with a high magnetization value that results in the characteristic superparamagnetism of the nanoparticles [1]. SPIO nanoparticles are good contrast agents for use in MRI, but this modality does not readily provide quantitative distribution data. SPIO nanoparticles have been labeled with ^{59}Fe to allow quantitative biodistribution studies based on dissection of animal tissues [6]. However, the decay properties of ^{59}Fe are not favorable for nuclear medical imaging [7]. A radionuclide commonly used in clinical nuclear medicine is ^{111}In. It emits gamma-rays of energies 0.17 and 0.25 MeV, which combine the features of good ability to penetrate the human body, with efficient detection using nuclear medicine imaging instruments [7]. Also, the half-life of ^{111}In is 2.8 days, which offers a reasonable "shelf-life" in the radiopharmacy with acceptably rapid clearance from the patient by physical decay after imaging studies have been completed. Furthermore, the electron-capture mode of decay avoids the higher radiation doses to tissues associated with nuclides that emit beta particles.

Nanoparticles have shown a tremendous potential in various biomedical applications. The ease of combining different imaging agents in a single platform, by means of using nanoparticles, paves the way for multimodal imaging using a single agent. Each imaging component can provide complimentary information based on which, the diseased sites can be diagnosed and detected efficiently with greater accuracy, both qualitatively and quantitatively. However, it is imperative to study the stability and integrity of these imaging agents conjugated to nanoparticles via different interactions (ionic interactions, covalent conjugations, etc.). The co-localization of different imaging agents in similar organs or diseased site can provide limited information on stability of these multimodal imaging platforms. Thus, it is very critical to assess the stability of these interactions once nanoparticles are administered in systemic circulation.

The objective of this manuscript is to study the *in vivo* integrity of ligand stabilized SPIO in systemic circulation. We adapted the synthetic approach of Jain et al. [8] to prepare oleic acid-stabilized hydrophobic iron oxide nanoparticles, which were further stabilized with different phospholipids to impart aqueous dispersibility for *in vivo* administration in mice. We incorporated different radiolabels: (a) ^{111}In using DMPE-DTPA on SPIONs platform for multimodal imaging; (b) ^{59}Fe to label the iron oxide core and, (c) ^{14}C to label the oleic acid. All three

☆ Funding: Office of Science (BER) U.S. Department of Energy, NSF DGE 0965843, the Center for Integration of Medicine and Innovative Technology under CIMIT 13-1087, and the Electronics Materials Research Institute at Northeastern University.
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http://dx.doi.org/10.1016/j.nucmedbio.2014.08.014
0969-8051/

radiolabels were studied to quantify the distribution of each component and correlate the integrity of SPIONs in circulation. We expected that after intravenous administration in mice, the labeled nanoparticles would be accumulated by reticuloendothelial cells in the liver and spleen, and therefore that if the ^{111}In remained associated with the nanoparticles, the radioactivity should be found predominantly in these organs. Furthermore, if the nanoparticles remain intact in the blood systemic circulation after administration, then all the components (iron oxide core, intermediate oleic acid layer and phospholipid-DTPA-^{111}In on the surface) should be delivered to various organs to the same degree. We believe that since the core of nanoparticles is labeled with a radioisotope of iron (^{59}Fe), and the oleic acid is labeled with a radioisotope of carbon (^{14}C), then the organ distributions of these nuclides after intravenous administration should be the same as that seen with ^{111}In-labeled SPIO nanoparticles. Furthermore, the biodistribution of ^{111}In after administration of ^{111}In-labeled nanoparticles should be distinct from the biodistribution patterns of other ^{111}In species (^{111}In-citrate, DTPA-^{111}In, and DMPE-DTPA-^{111}In). While our studies were in progress, Freund et al. [6] reported that ^{14}C-oleic acid label may be rapidly removed from the iron core after intravenous injection of similarly labeled SPIO nanoparticles, leading them to suggest that ^{14}C-oleic acid-stabilized nanoparticles require careful assessment before using them for in vivo studies [6]. This study provides further insight into the stability of these ligand-stabilized nanoparticles and how these self-assembled nanoformulations could be engineered in the future for efficient in vivo applications.

2. Materials and methods

2.1. Animals

Male Swiss Webster mice (Charles River Laboratories, Cambridge, MA) weighing 25–30 g were used for all in vivo studies. Mice were maintained at the animal facility of Division of Laboratory Animal Medicine (DLAM) on 12 h alternating light and dark periods, with access to food and water ad libitum. Mice were treated humanely in compliance with NIH guidelines for the use of laboratory animals, and according to a protocol approved by Northeastern University Institutional Animal Care and Use Committee (IACUC).

2.2. Materials

Iron (II) chloride tetrahydrate (99+%, Acros Organics) ($FeCl_2 \cdot 4H_2O$), iron (III) chloride hexahydrate (99+%, Acros Organics) ($FeCl_3 \cdot 6H_2O$) and ethanol (200 proof, USP/NF) were purchased from Fisher Scientific (NJ). Sodium chloride (ACS Reagent, 99+%), oleic acid (99+%), ammonium hydroxide solution (ACS reagent, 28.0–30.0% NH_3 basis), chloroform (ACS Reagent, 99.8+%), and HPLC grade water were purchased from Sigma-Aldrich (Saint Louis, MO). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-mPEG2000, CAS474922-77-5) and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-diethylenetriaminepentaacetic acid (DMPE-DTPA, CAS 384832-89-7) were purchased from Avanti Polar Lipids (Alabaster, AL). ^{111}In radionuclide indium citrate, ^{59}Fe radionuclide ferric chloride, [1^{14}C] oleic acid, Solvable™ and Ultima Gold™ XR were purchased from Perkin Elmer (Waltham, MA).

2.3. Preparation of SPIO nanoparticles

The nanoparticles were prepared from ferric and ferrous chloride by co-precipitation and stabilization with oleic acid with slight modifications from the published procedure [8,9]. Briefly, 15 mL of 0.1 M $FeCl_2 \cdot 4H_2O$ with 30 mL of 0.1 M $FeCl_3 \cdot 6H_2O$ was mixed in a round bottom flask equipped with a thermometer. The molar ratio of Fe^{2+} and Fe^{3+} was kept at 1:2.2. The solution was bubbled with argon and stirred for 20 min in the chemical hood before heating. When the temperature reached 80 °C, 3 mL of 5 M NH_4OH was added drop-wise, the clear pale yellow–green solution immediately turned dark brown–black indicating the formation of iron oxide nanoparticles. At that point, 100 mg of oleic acid was added to the mixture. The heating continued for another 30 min while the temperature was maintained at 80 °C. The sample was then allowed to cool to room temperature (RT). The resulting SPIO nanoparticles were separated from the solution using a strong magnet and the particles were washed with ethanol twice, dried under argon, and re-dispersed in chloroform. Using a 10 mL syringe (Fisher Scientific, NJ), the re-dispersed SPIO nanoparticles were filtered through PVDF 0.45 μm filters (Millipore™ Millex™ Sterile Syringe Filters, Fisher Scientific, NJ) to remove large aggregates, dried under argon, carefully weighed, and re-dispersed in chloroform to produce a 20 mg/mL suspension of SPIO nanoparticles.

PEGylated SPIO nanoparticles were prepared by the rehydration method [8,9]. In brief, 10 mg of DSPE-mPEG2000 and 2.5 mg of SPIO nanoparticles were mixed in chloroform. The organic solvent was removed by using a rotary evaporator to complete dryness. The film was then warmed in 80 °C water for 1 min and rehydrated using 1 mL of HPLC grade water. The film was placed in a sonicating bath for 20 min to obtain a suspension of PEGylated SPIO nanoparticles. Uncoated nanoparticles were removed by applying an external magnet to the bottom of the tube, and the supernatant was removed to a fresh vial.

2.4. Characterization of nanoparticles

The particle size distribution and surface charge (zeta potential) of the PEGylated SPIO nanoparticles were determined by dynamic light scattering (DLS) measurement with Brookhaven Instrument's 90 Plus particle size analyzer (Holtsville, NY) [12,13]. The particle size was determined at a 90° angle and at 25 °C, and the average count rate was adjusted in the range of 100–500 kcps by proper dilution (10 μL of the PEGylated SPIO nanoparticles solution was diluted in 2 mL deionized water). The mean number and diameter of the nanoparticles were obtained on MSD distribution mode. The average zeta potential values were determined based on the electrophoretic mobility using the Smoluchowski–Helmholtz equation. Each sample was analyzed five times with 10 cycles each time, and an average value was obtained from the five measurements. The PEGylated SPIO nanoparticles were stored at 4 °C and their stability was assessed on day 2, day 3, day 4, day 5, day 6 and day 7.

A JEOL 100-X transmission electron microscope (Peabody, MA) accelerating at 80 KV was utilized for analyzing the structure of the iron core of the PEGylated SPIO nanoparticles [14]. 10 μL of the PEGylated SPIO nanoparticles solution was diluted in HPLC grade water, placed on the carbon-coated copper grids, and allowed to air-dry at RT.

2.5. Radiolabeling PEGylated SPIO nanoparticles with ^{111}In

To radiolabel PEGylated SPIO nanoparticles with ^{111}In, the nanoparticles were synthesized as described above along with addition of 0.5 mol% of DMPE-DTPA to the lipid film [10,11]. 1 mL of DMPE-DTPA containing SPIO nanoparticles was incubated for 1 h with 10 μL of ^{111}In citrate solution (diluted to approximately 8–10 μCi by using 0.5 M HCl) at RT, to allow for the transchelation of ^{111}In from a weak citrate complex into a strong DTPA complex. The radioactivity was measured in an ionization chamber (CAL/RAD MARK IV, Fluke Biomedical, Cleveland, OH) with a setting of 676. The unbound ^{111}In was removed by a 30 kilodalton cutoff microcentrifuge filter (Amicon Ultra 0.5 mL centrifugal filters, Sigma-Aldrich, St. Louis, MO) at 14,000 rpm for 15 min at 4 °C for 3 times. The radiolabeled PEGylated SPIO nanoparticles were recovered from the filter using 1.5 mL of 0.9% NaCl solution.