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^{18}F Labeled Nanoparticles for *in Vivo* PET-CT Imaging

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Abstract

We report the synthesis and *in vivo* characterization of an ^{18}F modified trimodal nanoparticle (^{18}F -CLIO). This particle consists of cross-linked dextran held together in core-shell formation by a superparamagnetic iron oxide core and functionalized with the radionuclide ^{18}F in high yield via “click” chemistry. The particle can be detected with positron emission tomography, fluorescence molecular tomography, and magnetic resonance imaging. The presence of ^{18}F dramatically lowers the detection threshold of the nanoparticles, while the facile conjugation chemistry provides a simple platform for rapid and efficient nanoparticle labeling.

INTRODUCTION

Polymer-coated metal core nanoparticles have emerged as important platforms for a variety of biological applications such as bioseparation, sensing, molecular imaging, and drug delivery. Examples of such materials include carbohydrate-coated iron oxide nanoparticles (1–3) for magnetic applications, synthetic polymer coated cores for molecular imaging (4,5), gold nanoshells for therapy, or biocompatible quantum dots (6,7). Magnetic nanoparticles, in particular, have received much attention, as they were shown to be biocompatible (8) and have low clinical toxicity (9), and subsequently were among the first nanomaterials tested in clinical trials (10). While native carbohydrate-coated preparations primarily target macrophages, monocytes, and other phagocytic cells *in vivo*, conjugation of affinity ligands has been used to image other molecular targets *in vivo* (11,12). We have recently shown that small molecule modifications (13) can yield uniquely targeted nanoparticles with low toxicities (14). Despite these advances, the *in vivo* detection threshold of nanoparticles via magnetic resonance is generally in the micromolar range requiring an estimated ~200–700 mg (Fe) for a 70 Kg patient.

One avenue to substantially decrease the detection threshold and therefore the clinical dose is to label nanoparticles with positron emitters and detect them by position emission tomography (PET) imaging. Recently, we reported the use of a ^{64}Cu labeled trireporting nanoparticle that could be used at ~100 times lower doses for PET imaging as compared to MRI and was employed to image macrophages in inflammatory atherosclerotic plaques (15). This material was based on aminated cross-linked dextran iron oxide (CLIO) decorated with diethylene triamine pentaacetic acid (DTPA) to transchelate ^{64}Cu . While we showed proof of principle, ^{64}Cu is not widely available, complicating clinical day-to-day on-demand syntheses. On the contrary, ^{18}F is a widely available PET isotope that is produced daily in cyclotrons of

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most major hospitals and distribution centers. ^{18}F has additional advantages over ^{64}Cu such as higher sensitivity, shorter half-life, and the ability of irreversible covalent bonding. Despite these advantages, the addition of ^{18}F to functionally complex materials is chemically challenging and is a major reason for the absence of reports of ^{18}F iron nanoparticles. Here, we report a facile and straightforward synthesis method for an ^{18}F trimodal nanoparticle (^{18}F -CLIO, Figure 1) and demonstrate its use for in vivo PET imaging. In this work, we have used “click” chemistry (16,17), the copper-catalyzed azide–alkyne cycloaddition, to introduce ^{18}F onto an azide-modified nanoparticle.

The introduction of ^{18}F to functionally complex molecules has been an outstanding problem in nuclear chemistry. Though there are many reported conjugation methods, most suffer from the requirement of harsh coupling conditions, long reactions times, multistep protocols, or poor overall yields. A number of ^{18}F labeling methods have been described and are conceivable for nanoparticle labeling. One such method, nucleophilic aromatic substitution, used to prepare *N*-succinimidyl 4- ^{18}F fluorobenzoate and 4- ^{18}F fluorobenzaldehyde, either requires multiple steps post-labeling to obtain an amine-reactive species for the former or produces a volatile compound difficult to concentrate for subsequent bimolecular reactions in the case of the latter (18,19). In contrast, “click” chemistry, specifically copper-catalyzed azide–alkyne cycloaddition, has proven to be an extremely robust, mild, conceptually simple, and chemiselective method to conjugate function to complex macromolecules and biomolecules. Recently, there have been a variety of reports demonstrating that the “click” reaction can be used efficiently and mildly to conjugate an ^{18}F label to a variety of peptides and small molecules (20,21). These reports motivated us to explore the application of “click” chemistry to modify nanoparticles with ^{18}F .

MATERIALS AND METHODS

Materials

All reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. 1-Amino-11-azido-3,6,9-trioxundecane was purchased from Toronto Research Chemicals and used as received. Sodium [^{18}F]-fluoride ($\text{Na}[^{18}\text{F}]\text{F}$) in ^{18}O -enriched water was purchased from PETNET (Woburn, MA). The well-characterized nanoparticle MION (MGH-CMIR, Charlestown, MA) served as starting material (2). The dextran coating of the nanoparticle was cross-linked with epichlorin hydrin, aminated, and labeled with near-infrared fluorochrome Vivotag-680 (VT680, VisEn Medical, Woburn, MA) (22). The ratio of VT680 per nanoparticle was approximately 5 dye moieties/particle. Preparative-scale high-performance liquid chromatography (HPLC) purification was achieved using a Machery-Nagel Nucleodur C18 Pyramid 250×10 mm Vario-Prep column (60:40 0.1% trifluoroacetic acid (v/v) in water–acetonitrile (AcCN) at $5.5 \text{ mL} \cdot \text{min}^{-1}$) with a 254 nm UV detector and radiodetector connected in series. Analytical HPLC was performed employing a Grace VYDAC (218TP510) C18 reversed-phase column (eluent 0.1% TFA (v/v) in water and AcCN; gradient: 0–17 min, 5–60% B; 17–21 min, 60–95% B; 21–24 min, 95% B; 24–25 min, 95–5% B; 25–30 min, 5% B; $2 \text{ mL} \cdot \text{min}^{-1}$) with a dual-wavelength UV–vis detector and a flow-through gamma detector connected in series. HyperSep C18 cartridges were purchased from Thermo Electron (Bellefonte, PA) and Sep-pak VAC Alumina-N cartridges from Waters (Milford, MA).

Synthesis of ^{18}F -PEG₃

The preparation of the 2-(2-(2-(prop-2-ynoxy)ethoxy)ethoxy)ethyl tosylate (20) precursor started from the monopotassium salt of triethylene glycol, which was alkylated with propargyl bromide. This propargyl PEG₃ was treated with tosyl chloride in the presence of triethyl amine. 3-(2-(2-(2- ^{18}F -Fluoroethoxy)-ethoxy)ethoxy)prop-1-yne (^{18}F -PEG₃) was prepared using Synthra RN Plus automated synthesizer (Synthra GmbH, Hamburg, Germany) operated by

Explora View software in an average time of 40 min. The target well was charged with Na [^{18}F]F, n.c.a., (~1850 MBq, 50 mCi) in H_2^{18}O (150 μL), 150 μL of a 74 mM K_2CO_3 solution in water, and 250 μL of 49 mM Kryptofix 2.2.2 in AcCN. The synthesizer reagent vials were filled as follows: A2 with AcCN (350 μL), A3 with tosylate **2** (1.0 mg, 19.2 μmol) in DMSO (400 μL), A5 with DMSO (50 μL), and B2 with H_2O (900 μL). The $\text{Na}[^{18}\text{F}]\text{F}/\text{K}_2\text{CO}_3/\text{K}2.2.2$ solution was transferred to Reaction Vessel #1 and dried by azeotropic distillation of the acetonitrile/water solution by heating to 60 $^\circ\text{C}$ under reduced pressure and a flow of argon to achieve ~310 mbar for 2 min followed by 98 $^\circ\text{C}$ and 270 mbar for 3 min. Reaction Vessel #1 was cooled to 50 $^\circ\text{C}$, tosylate **2** in DMSO (400 μL) added, and the reaction vessel pressurized to 2000 mbar and heated to 110 $^\circ\text{C}$ for 15 min. After cooling to 30 $^\circ\text{C}$, the mixture was filtered through an Alumina-N cartridge (100 mg, 1 mL, Waters) into Reaction Vessel #2. The Alumina-N cartridge was washed with DMSO (50 μL), and the combined filtrates were diluted with water (900 μL). This solution was subjected to preparative HPLC purification. ^{18}F -PEG₃ was collected (t_R = 4.9 min) in 3–4 mL of solvent and isolated by C18 solid-phase extraction. Elution of the collected material with CH_2Cl_2 (600 μL) to give ^{18}F -PEG₃ in 57% average decay-corrected radiochemical yield (RCY). Analytical HPLC demonstrated >99% radiochemical purity of ^{18}F -PEG₃, Figure 2.

Preparation of Azido-PEG Modified CLIO

1-Amino-11-azido-3,6,9-trioxaundecane (218 mg, 1 mmol) and glutaric anhydride (114 mg, 1 mmol) were dissolved in 1 mL methylene chloride and refluxed for 1 h. Evaporation of solvent yielded 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid quantitatively, verified by LRMS-ESI $[\text{M} + \text{H}]^+$ m/z calc. $[\text{C}_{13}\text{H}_{25}\text{N}_4\text{O}_6]^+$ 333.17, found 333.3. The azido-acid (33 mg, 0.1 mmol) was stirred for 3 h in 0.3 mL AcCN with 8 μL of pyridine and 26 mg of disuccinimidylcarbonate. The AcCN was evaporated, and the resulting crude *N*-hydroxysuccinimide ester was used directly with amine-terminated iron oxide nanoparticles. The aminated cross-linked dextran iron oxide (CLIO) nanoparticle was prepared as previously described (23). Nanoparticles were approximately 30 nm in diameter and contained on average 40 primary amines. Aminated CLIO was functionalized first with NHS modified VT680 (VisEn Medical, Woburn, MA) such that there were approximately 5 molecules of VT680 per particle. The remaining amines were capped with the NHS ester of 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid to give the CLIO-azide. In a typical coupling procedure, 0.4 mg of amine-terminated CLIO was dissolved in 0.5 mL of 0.1 M sodium bicarbonate buffered to a pH of 8.5. To this solution was added 40 μL of DMSO containing 1 mg of the azido *N*-hydroxysuccinimide ester. The solution was stirred at room temperature for 2 h, purified by centrifuge filtration, and stored in PBS buffered to pH 7.4.

Conjugation

^{18}F -PEG₃ in 50–100 μL water added to the azido-CLIO (50 μL of a 400 $\mu\text{g}/100 \mu\text{L}$ 1 $\times$ PBS (phosphate buffered solution)) was subjected to water-soluble copper-catalyzed 1,3-dipolar cycloaddition reaction conditions: 40 μL 10 mM $\text{Cu}^{(\text{II})}\text{SO}_4$ /bathophenanthrolinedisulfonic acid disodium salt (BPDS) in distilled water/DMSO (3:1) and 40 μL 100 mM Na-ascorbate in distilled water (24). The reaction mixture was incubated for 40 min at 40 $^\circ\text{C}$ and filtered to remove the ^{18}F -CLIO from unreacted ^{18}F -PEG₃. ^{18}F -CLIO was washed with 1 $\times$ PBS until no activity was collected in the filtrate (>1 μCi). HPLC analysis demonstrated >99% radiochemical purity of ^{18}F -CLIO (Figure 2). The ^{18}F -labeled nanoparticle was suspended in DPBS (Dulbecco's phosphate buffered solution with Ca^{2+} and Mg^{2+}).

Phantom Experiment

To compare the sensitivity of nanoparticle detection with MRI and PET, ^{18}F -CLIO was diluted in 2% low melting agarose to concentrations ranging from 0.005 to 10 $\mu\text{g Fe}/\text{mL}$ and added

to wells in a precast gel. PET-CT imaging of 2 gels was followed by MRI using a 7 T Pharmascan magnet (Bruker, Billerica, MA) and employing a T2* weighted gradient echo sequence (TE 140 ms, matrix 128×128 , FOV $60 \times 40 \text{ mm}^2$, slice thickness 0.8 mm).

Mice

Inbred male BALB/c (B/c, H-2d) mice (8–12 weeks old) were obtained from Jackson Laboratory (Bar Harbor, ME). The mice were maintained at the animal facilities of Massachusetts General Hospital, accredited by the American Association of Laboratory Animal Care. All experiments conformed to animal care protocols approved by the institutional review board.

PET Imaging

Mice were imaged with PET-CT one hour after injection of $998 \pm 74 \text{ } \mu\text{Ci}$ of ^{18}F -CLIO ($n = 6$). This concentration was about 1 order of magnitude lower than previously used for MRI (10–20 mg Fe/kg). PET-CT was performed on an Inveon system (Siemens Preclinical Solutions). PET scans were terminated after 600 million events were recorded, with a typical acquisition time of 45 min. Spatial resolution of PET imaging approximated 1 mm isotropic. Acquisition coincidence data were rebinned using Fourier rebinning in 3D, and the resulting sinogram was reconstructed using an ordered subsets expectation maximization (OSEM) algorithm. For quantification of standard uptake values (SUV), images were reconstructed using 2D filtered back projection (FBP). The CT X-ray source was used with a power of 80 kVp and 500 μA and an exposure time of 370–400 ms. The effective 3D resolution was 90 μm isotropic. Typically, 360 projections were acquired. The reconstruction protocol was set to interpolate bilinearly, used a Shepp-Logan filter, and signal intensity was scaled to Hounsfield units. To achieve appropriate intravascular contrast, iopamidol 61% (Bracco Diagnostics, Princeton, NJ) was continuously infused via tail vein at a rate of 65 $\mu\text{L}/\text{min}$ for the time of CT acquisition, which was approximately 7 min. Fused images were converted into DICOM format and analyzed using OsiriX shareware (Geneva, Switzerland, www.osirix-viewer.com) by placing volumes of interest in the various organs for calculation of target-to-background ratios. Standard uptake values (SUV) were calculated as (tissue activity/volume of tissue)/(body activity/body weight).

Pharmacokinetic Analysis

Blood half-life data obtained from the heart (blood pool organ) were fit to a biexponential model, and the half-life is reported as the weighted average of the distribution and clearance phases. For fitting permeability surface area product (PS/V) values, the average concentration of activity (decay corrected) in a region of interest determined by CT was measured for the time series. The interstitial concentration was obtained by subtracting the blood contribution using the plasma fraction for each organ. Assuming an interstitial void fraction of 0.05 for these particles, PS/V was fit using the plasma clearance and specific organ data. Due to the early imaging sequence with ^{18}F , clearance was assumed to be negligible for all organs (uptake only) except the lung. The lung had similar low levels of activity as the kidney but demonstrated decreasing signal at later time points. Here, a value for lymphatic clearance was assumed to obtain a reasonable fit.

RESULTS

3-(2-(2-(2- ^{18}F -Fluoroethoxy)ethoxy)ethoxy)prop-1-yne (^{18}F -PEG₃) was prepared in 57% average decay-corrected radio-chemical yield from an alkynyl tosylate (see Supporting Information for full experimental details). ^{18}F -PEG₃ is prepared in one step and when compared with 4- ^{18}F fluorobenzaldehyde is relatively nonvolatile. This quality allows concentration of ^{18}F -PEG₃ to almost dryness, thus increasing the yield of the bimolecular

second step. “Click” reaction with an azide-terminated CLIO yielded ^{18}F -CLIO with a decay-corrected yield of 58%. Both ^{18}F -PEG₃ and ^{18}F -CLIO were analyzed by HPLC to determine radiochemical purity. Following preparative HPLC purification of ^{18}F -PEG₃ and microcentrifuge filtration of ^{18}F -CLIO, both were observed to be >98% pure (Figure 2). Average total ^{18}F -CLIO synthesis time was 120 min from the start of radiolabeling of ^{18}F -PEG₃. ^{18}F -CLIO had an average specific activity of 1.1 mCi/0.1 mg of nanoparticles. Control experiments using nanoparticles without azides or the absence of the copper catalyst failed in demonstrating the chemoselectivity of the conjugation.

The detection threshold of ^{18}F -CLIO suspended in the agar phantom was 0.025 $\mu\text{g Fe/mL}$ for PET-CT imaging, approximately 200 times lower than by T2*-weighted MR imaging (Figure 3). This is an improvement from ^{64}Cu -labeled nanoparticles of comparable specific activity. The increased sensitivity was expected due to the increased branching ratio for the decay of ^{18}F over ^{64}Cu (25). As previously determined, the lowest observed concentration of the nanoparticles by MRI was 5 $\mu\text{g Fe/mL}$ (15), demonstrating a PET detection threshold 200 times more sensitive than MRI at current specific activities. It is expected that optimization of reaction yields will further decrease the PET detection threshold.

Purified ^{18}F -CLIO was injected into the tail vein of 3 mice, and dynamic PET imaging was performed to determine organ biodistribution and blood clearance over time. Figure 4 summarizes one imaging series in which data were collected over the course of 16 h after intravenous injection of ^{18}F -CLIO and shows images acquired at 2, 7, and 16 h. Initially, high sustained activity was detected in the blood pool, demonstrated by high PET signal in the cardiac chambers. Over time, this signal declined, while an increase of activity was observed in the liver and spleen. Using compartmental analysis, the uptake rate in the liver was much higher than uptake by the kidney (~4-fold, Table 1), and some of the renal activity may have indeed been due to the close proximity of the region of interest to the liver. The low renal and bladder uptake emphasizes that ^{18}F -CLIO is stable in vivo, and the tracer is not excreted via the kidneys. Taken together, these PET data follow the typical pharmacokinetics of CLIO, showing redistribution of the blood pool signal to the liver and lymphatic organs over the course of several hours (Figure 4D–F).

In vivo dynamic PET imaging showed very high signal-to-noise ratios for injected ^{18}F -CLIO. As summarized in Figure 4 and Figure 5, the nanoparticle has a vascular half-life of 5.8 h in mice and is subsequently internalized into macrophages of liver, spleen, and phagocytic cells of other lymphatic organs. The nanoparticle is biodegradable and had previously been shown to be broken down into elemental components in weeks–months time frames (8). There was negligible urine and/or bowel excretion (<2%) over 24 h. The iron is metabolized and is reused in heme synthesis (8). The 10 kDa dextran coating is slowly excreted as determined by ^{14}C labeling studies. While long-term toxicity experiments have not been conducted for this agent, an adverse reaction is not expected due to the substantially lower iron doses administered than those of previous studies that demonstrated no adverse acute, subacute, or chronic effects (8, 15).

DISCUSSION

A major future application of the above approach is to use nanoparticle platforms for targeted molecular imaging. Nanoparticles allow multivalent attachment of small affinity ligands, vastly increasing avidity. This together with optimized pharmacokinetics of parent materials would allow a modular approach to rapidly building and testing such materials. Our approach

Supporting Information Available: Methods and Materials. This material is available free of charge via the Internet at <http://pubs.acs.org>.

for ^{18}F labeling of such materials is yet another step to rapidly test their efficacy in vivo. The ability to use targeted nanomaterials at 2–4 orders of magnitude lower doses than those required for MR imaging will likely make PET imaging an important platform for clinical molecular imaging.

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Figure 1. Preparation of ${}^{18}\text{F-CLIO}$. (A) Derivatization of primary amines on CLIO-VT680 with the NHS ester of 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid followed by chemoselective "click" of ${}^{18}\text{F-PEG}_3$ radiotracer. (B) Schematic of ${}^{18}\text{F-CLIO}$.

Figure 2.
HPLC analysis of purified ^{18}F -PEG₃ and ^{18}F -CLIO demonstrating >98% purity.

Figure 3. PET imaging of 2 agar phantoms with decreasing concentrations of ^{18}F -CLIO ($\mu\text{g Fe/mL}$).

Figure 4.

Dynamic PET/CT imaging of BALB/C mouse injected with ^{18}F -CLIO. Fused PET/CT coronal images at 2 h (A), 7 h (B), and 16 h (C) postinjection of ^{18}F -CLIO. PET only coronal images at 2 h (D), 7 h (E), and 16 h (F) postinjection of ^{18}F -CLIO. CT only coronal image (G). Three-dimensional rendering of fused PET-CT images at 2 h (H) and 16 h (I) postinjection. Arrow (green) indicates blood pool region of interest (ROI) and asterisk indicates liver ROI.

Figure 5. Activity density vs time curves for blood pool of the heart and liver. Blood half-life ($t_{1/2}$) was calculated to be 5.8 h.

Table 1Permeability Surface Area Product (PS/V) for Major Organs^a

organ	PS/V (10^{-5} /s)	clearance
liver	32.9	-
spleen	20.4	-
kidney	8.64	-
lung	33.2	0.000089/s

^aValues were fit to ROI data from serial PET imaging.