JRMP Journal of Radiopharmaceuticals and Molecular Probes Vol. 4, No.2, 2018
J Radiopharm Mol Prob 2018;4(2): 45-50
ISSN 2384-1583(Print) / ISSN 2508-3848(Online)

ORIGINAL ARTICLE

https://doi.org/10.22643/ JRMP.2018.4.2.45

Synthesis of oleyl-4[^{131}I]-iodobenzoate for long-term cell trafficking

Hak Rim Jeon[1], Woonghee Lee[1], Jieun Oh[1], Yong Jin Lee[2], Jeongsoo Yoo[1*]

[1] Department of Molecular Medicine, BK21 Plus KNU Biomedical Convergence Program, School of Medicine, Kyungpook National University, Daegu, South Korea
[2] Department of RI-Convergence Research, Korea Institute of Radiological and Medical Sciences, Seoul, South Korea

ABSTRACT

Great efforts are currently devoted to the development of new approaches for the labeling of cells using appropriate radionuclides. While fluoride-18 and copper-64 have been extensively studied as short-term and intermediate-term trafficking agents, iodide was studied less intensely. Here, we report a new cell labeling agent labeled with ^{131}I , [^{131}I]oleyl-4-iodobenzoate ([^{131}I]OIB) for long-term cell trafficking. A precursor of [^{131}I]OIB was obtained in two steps, with the yield of 35%. The radiochemical yield of [^{131}I]OIB was over 50%. While [^{131}I]OIB could label different cells, L6 cells showed the highest cell-labeling efficiency. The [^{131}I]OIB-labeled L6 cells were imprinted into a rat heart, and then monitored noninvasively for 2 weeks by gamma camera imaging. We conclude that [^{131}I]OIB is a good candidate molecule for a long-term cell trafficking agent.

Key Word: Radiotracer, Cell trafficking agent, I-131, Gamma camera imaging

Introduction

Gamma camera imaging is a powerful non-invasive molecular imaging technique that provides functional information on physiological, biochemical, and pharmacological processes and human.[1–3] The possibility to observe molecular interactions in a living organism and to determine absolute physiological parameter values places gamma camera imaging in a unique position among other molecular imaging techniques. Fluorine-18 (^{18}F) is an almost ideal radionuclide for positron emission tomography (PET) imaging because of ease of production and favorable physical properties, such as a half-life of 109.8 min and low ß$^{+}$ energy (0.64 MeV).[4,5] Although ^{18}F-labeled radiotracers, such as 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]-FDG), hexadecyl-4-[^{18}F]fluorobenzoate ([^{18}F]-HFB) and 4-[^{18}F]fluorobenzamido-N-ethylamino-maleimide ([^{18}F]-FBEM), were tested as cell trafficking imaging agents and metabolic markers in biomedical research as well as in clinical practice, its relatively short half-life has prompted development of other potential radiotracers of longer half-life.[6-8] Copper-64 (^{64}Cu) has longer half-life ($t_{1/2}$ = 12.7 h) compared to ^{18}F. Cell labeling with ^{64}Cu had also been accomplished using ^{64}Cu-pyruvaldehyde-bis(N^{4}-methylthiosemicarbazone) ([^{64}Cu]Cu-PTSM) and

Received: November 27, 2018 / Revised: December 15, 2018 / Accepted: December 18, 2018

Correspnding author : Jeongsoo Yoo, Department of Molecular Medicine, BK21 Plus KNU Biomedical Convergence Program, School of Medicine, Kyungpook National University, 680 Gukchaebosang-ro, Joon-gu, Daegu 41944, South Korea
Tel: +82-53-420-4947, Fax: +82-53-426-4944, Email: yooj@knu.ac.kr

^{64}Cu-hexadecyl-1,4,7,10-tetraazacyclododecane-tetraacetic acid-benzoate ([^{64}Cu]Cu-DOTA-HB).[9-11] However, the in vivo cell trafficking using ^{64}Cu-labeled trafficking agents were feasible up to only few days. And, it is not suitable for long-term cell trafficking. The choice of the appropriate radionuclide is among the most important aspects of the design and application of novel gamma camera radiotracers. The physical half-life of the radionuclide should reflect the time frame of the biological process to be studied. High yields of several gamma camera radiotracers with different physical half-lives have been obtained.[12-16] Prominent examples of gamma camera radionuclides with long half-lives include iodine-131 ($t_{1/2}$ = 8 d).

Recently, halogen ^{131}I has become an attractive long-lived radionuclide for the design and synthesis of novel gamma camera radiotracers. Its convenient 8 d half-life allows extended radiosynthesis protocols and long-term gamma camera imaging studies. Furthermore, labeling chemistry for ^{131}I is well established, and a wide variety of compounds have been labeled for the purpose of molecular imaging using gamma camera.

Here, we report a simple method for cell labeling with iodine-131, which involves a single-step radiochemical synthesis of a lipophilic long-chain ester, oleyl-4-[^{131}I]-iodobenzoate ([^{131}I]OIB), which is efficiently and quickly intercalated into the cellular membranes in a fashion similar to that of fluorescent dyes used for cell labeling. The iodine-131 label is well retained both in vitro and in vivo, providing a labeling method suitable for in vivo gamma camera imaging studies of cell trafficking.

Materials

All reagents and solvents were obtained from Sigma-Aldrich. Silica gel used in flash chromatography was purchased from Aldrich. Reaction progress was monitored by analytical thin-layer chromatography (Merck) with visualization using short-wave UV light (254 nm). ^{1}H- and ^{13}C-NMR spectra were obtained using Bruker DPX 300 instrument, and data are expressed relative to TMS as an internal standard. All animal experiments were conducted in compliance with the Animal Care and Use Committee requirements of Kyungpook National University.

Synthesis of OIB

Oleylalcohol (2.7 mL, 9.31 mmol) and triethylamine (2.25 mL) were added to a mixture of 4-iodobenzoyl chloride (2.65 g, 9.94 mmol) and dichloromethane (130 mL). The reaction mixture was stirred at room temperature overnight. Volatiles were removed under reduced pressure and the residue was purified on column chromatography to yield OIB (2.80 g, 60%). ^{1}H NMR δ 7.78 (4H, ABq, J = 8.48 Hz), 5.36 (2H, m), 4.32 (2H, t, J = 8 Hz), 2.04 (4H, m), 1.77 (2H, m), 1.28–1.44 (22H, m), 0.88 (3H, m). ^{13}C NMR δ 14.55, 23.11, 26.41, 27.58, 27.63, 29.06, 29.61, 29.66, 29.74, 29.82, 29.95, 30.07, 30.13, 30.18, 32.32, 33.03, 65.76, 100.97, 130.14, 130.60, 130.84, 138.32, 166.46.

Synthesis of oleyl-4-tributyltinbenzoate

Hexabutyltin (1 mL, 1.39 mmol) and tetrakis(triphenyl-phosphine)palladium (0.02 g, 17.3 μmol) was added to a solution of OIB (0.46 g, 0.87 mmol) in toluene (150 mL), and the reaction mixture was refluxed under argon

until it turned black. Volatiles were removed under reduced pressure and the residue was purified by column chromatography to yield oleyl-4-tributyltinbenzoate (0.37 g, 61%). ^{1}H NMR δ 7.99 (2H, d, J = 8 Hz), 7.62 (2H, d, J = 8 Hz), 5.39 (2H, m), 4.33 (2H, t, J = 8Hz), 2.05 (4H, m), 1.8 (2H, m), 1.12–1.57 (22H, m), 0.91 (12H, m). ^{13}C NMR δ 10.03, 14.06, 14.52, 23.10, 26.46, 27.59, 27.62, 27.73, 29.44, 29.69, 30.14, 30.18, 32.30, 33.02, 65.36, 128.55, 130.65, 130.83, 136.76, 149.79, 166.48.

Radiochemical synthesis of [^{131}I]OIB

A mixture of oleyl-4-tributyltinbenzoate (50 μL, 1 mg/mL), 1 M HCl (50 μL), 3% H_2O_2, and [^{131}I]NaI was stirred at room temperature for 20 min. The reaction was quenched with saturated sodium bisulfate. The product was purified by HPLC (Luna C8 column, 5 μm, 4.6 × 50 mm; mobile phase: 95% acetonitrile/water, flow rate 1 mL/min). Volatiles were removed under reduced pressure. The isolated [^{131}I]OIB was dissolved in 10% DMSO/PBS.

Radiochemical yield was greater than 50% and the overall synthesis time was less than 2 h. Radiochemical purity, as determined by analytical HPLC, was >95% [Luna C5 (5 μm, 4.6 × 50 mm) column fitted with UV (254 nm) and radioactivity detectors; mobile phase: 95% acetonitrile/water and flow rate of 1 mL/min].

Cell labeling with [^{131}I]OIB

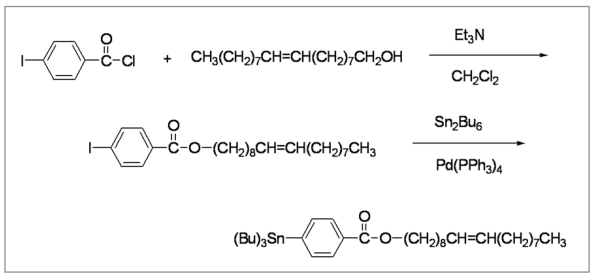
Attached L6, H9C2, and HeLa cells (4 ×10^5 cells) in 1 mL of serum-free DMEM medium were incubated at 37°C in 5% CO_2 for 1 h. Then, the serum-free DMEM medium was removed and the cells were washed twice with 1 mL of PBS, and re-suspended in 1 mL of serum-free DMEM medium. A solution of [^{131}I]OIB in 10% DMSO/PBS was added to a suspension of cells and the mixture was incubated at 37°C in 5% CO_2 for 90 min. After removing serum-free DMEM medium, the cells were washed twice with 1 mL of PBS. Then, 0.5 mL of trypsin-EDTA was added to the mixture, and xx of serum-free DMEM medium. After centrifugation (1000 rpm for 2 min), the supernatant was removed and the radioactivity of the isolated cell preparation was measured using a Capintec radioisotope calibrator.

Analysis of cells labeled with [^{131}I]OIB by gamma camera imaging

Gamma imaging was performed using GE infinia™. An SD rat labeled with [^{131}I]OIB was anesthetized with 1–2% isoflurane under 100% O_2 gas during injection and imaging. Images were acquired 1, 3, 4, 5, 6, and 14 d after injection of 300 μCi [^{131}I]OIB-labeled L6 cells into the open heart.

Rsults and Discussion

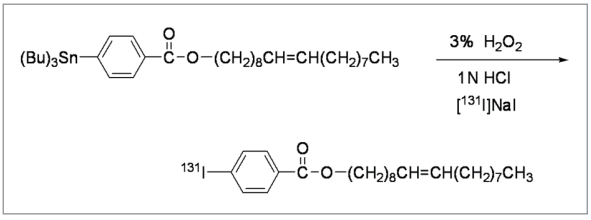
The precursor of [^{131}I]OIB was prepared in two steps. Treatment of 4-iodobenzoyl chloride with oleyl alcohol yielded OIB, followed by nucleophilic aromatic



Scheme 1. Synthesis of OIB and precursor oleyl-4-tributyltinbenzoate.

substitution with hexabutyltin, yielding a precursor of [^{131}I]OIB (yield of 35%) in two steps (Scheme 1).

[^{131}I]OIB was prepared by nucleophilic aromatic substitution of tin ester with [^{131}I]NaI (Scheme 2). The radiochemical yield of [^{131}I]OIB was greater than 50%. Radiolabeled [^{131}I]OIB was obtained with high



Scheme 2. Radiochemical synthesis of [^{131}I]OIB

radiochemical purity (93%) after HPLC purification (Figure 1).

The cells were labeled by stirring a suspension of [^{131}I]OIB and cells in PBS, followed by centrifugation and repeated washing of cells (Figure 2). The cell labeling yield of L6, H9C2, and HeLa cells with [^{131}I]OIB was 32 ± 6.9%, 27 ± 5.2%, and 22 ± 6.7%, respectively. These cell uptake data are comparable with those reported in other studies describing cell labeling with radioactive atoms.[6–11]

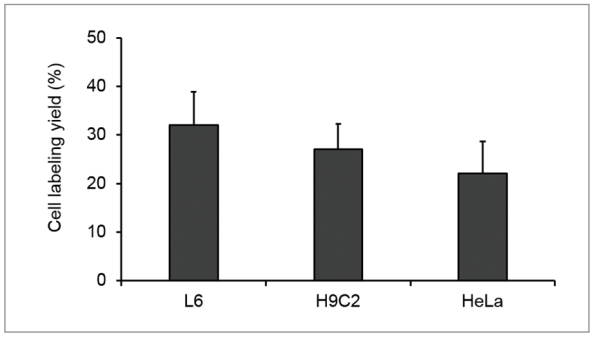


Figure 2. Cell labeling yield of L6, H9C2, and HeLa cells with [^{131}I]OIB.

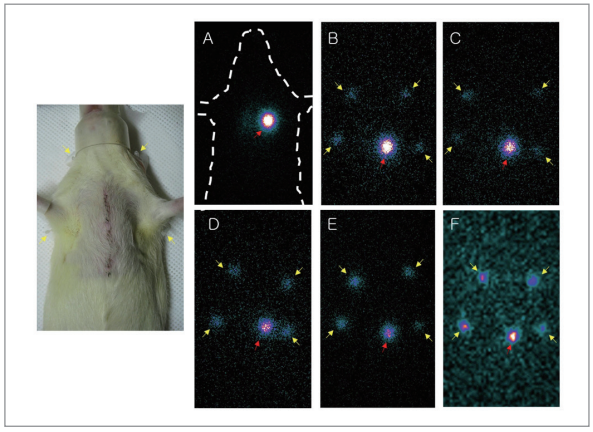


Figure 3. Gamma camera images of L6 cells labeled with [^{131}I]OIB after injection into an open rat heart. The images were acquired on days 1 (A), 3 (B), 4 (C), 5 (D), 6 (E), and 14 (F) after the injection. Red arrow, the heart; yellow arrow, fiducial markers

The concept of cell labeling with [^{131}I]OIB is similar to that of cell staining with fluorescent dyes.[12] [^{131}I]OIB

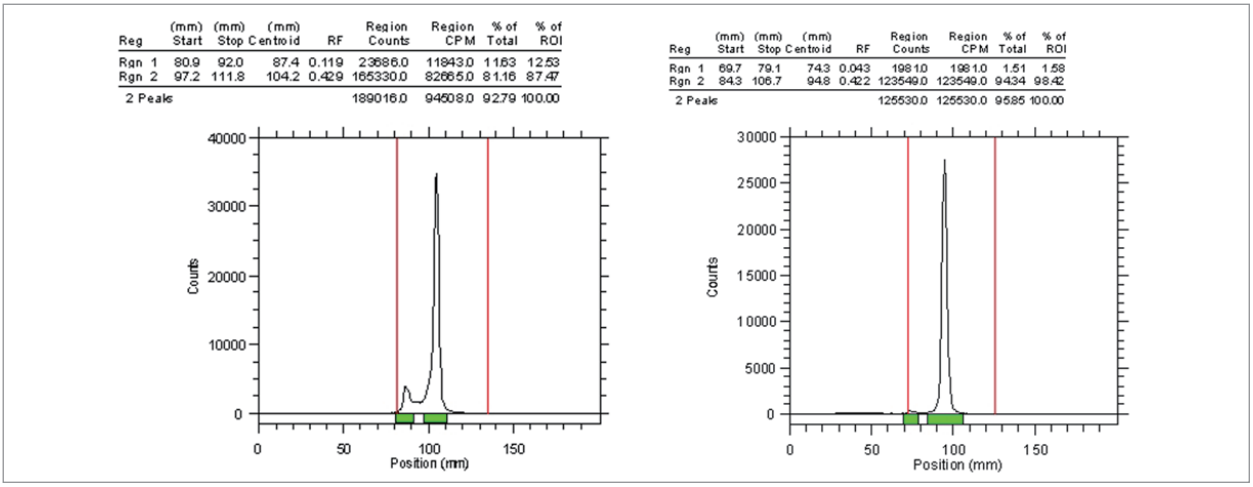


Figure 1. Radio–thin-layer chromatogram of [^{131}I]OIB (left: radiolabeling mixture; right: sample after HPLC purification).

is assumed to radiolabel the cells by cell membrane intercalation of the long alkyl chain.

The in vivo trafficking of cells labeled with [^{131}I] OIB was followed for 14 d by gamma camera imaging (Figure 3). Following an injection into the open heart, radioactivity was deposited into the heart, where it remained for 14 d. This clearly indicated that [^{131}I]OIB can be used as a new long-term cell trafficking agent.

Conclusion

We report a new cell labeling agent labeled with ^{131}I, [^{131}I]oleyl-4-iodobenzoate ([^{131}I]OIB). The L6 cells labeled with [^{131}I]OIB were inoculated into rat heart, then monitored successfully for two weeks by gamma camera imaging. Consequently, [^{131}I]OIB can be a good candidate of cell labeling agent for long-term cell trafficking.

Acknowledgments

This work was supported by an R&D program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (nos. 2017M2A2A6A02018506, 2016R1A2B4011546, and 2017R1D1A1B03033974) and a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea (no. HI17C0221).

References

1. Loudos G, Varvarigou A. Specified systems of high spatial resolution for tomographic gamma cameras; their applications and studies in Greece. *Hell J Nucl Med* 2006;9:126-132.
2. Ito S, Saze T, Ariga E, Deji S, Hirota M, Nishizawa K. ^{99m}Tc thyroid imaging system using multiple imaging plates. *Phys Med* 2009; 25:73-81.
3. Huang HK. Biomedical image processing. *Crit Rev Bioeng* 1981;5:185-271.
4. Essner R, Hsueh EC, Haigh PI, Glass EC, Huynh Y, Daghighian F. Application of an [^{18}F]fluorodeoxyglucose-sensitive probe for the intraoperative detection of malignancy. *J surg Res* 2001;96:120-126.
5. Franc BL, Mari C, Johnson D, Leong SP. The role of a positron and high-energy gamma photon probe in intraoperative localization of recurrent melanoma. *Clin Nucl Med* 2005;30:787-791.
6. Tamura M, Unmo K, Yonezawa S, Hattori K, Nakashima E, Tsukada H, Nakajima M, Oku N. In vivo trafficking of endothelial progenitor cells and their possible involvement in tumor neovascularization. *Life Sci* 2004;75:575-584.
7. Ma B, Hankenson KD, Dennis JE, Caplan AI, Goldstein SA, Kilbourn MR. A simple method for stem cell labeling with fluorine 18. *Nucl Med Biol* 2005;32:701-705.
8. Lacroix S, Egrise D, Van Simaeys G, Doumont G, Monclus M, Sherer F, Herbaux T, Leroy D, Goldman S. [^{18}F]-FBEM, a tracer targeting cell-surface protein thiols for cell trafficking imaging. *Contrast Media Mol Imaging* 2013;8:409-416.
9. Adonai N, Adonai N, Nguyen KN, Walsh J, Iyer M, Toyokuni T, Phelps ME, McCarthy T, McCarthy DW, Gambhir SS. Ex vivo cell labeling with ^{64}Cu-pyruvaldehyde-bis(N4-methylthiosemicarbazone) for imaging cell trafficking in mice with positron-emission tomography. *Proc Natl Acad Sci USA.* 2002;99:3030-3035.
10. Park JJ, Lee TS, Son JJ, Chun KS, Song IH, Park YS, Kim KI, Lee YJ, Kang JH. Comparison of cell-labeling methods with ^{124}I-FIAU and ^{64}Cu-PTSM for cell tracking using chronic myelogenous leukemia cells expressing HSV1-tk and firefly luciferase. *Cancer Biother Radiopharm* 2012;27:719-728.

11. Kim MH, Woo SK, Kim KI, Lee TS, Kim CW, Kang JH, Kim BI, Lim SM, Lee KC, Lee YJ. Simple Methods for Tracking Stem Cells with ^{64}Cu-Labeled DOTA-hexadecyl-benzoate. *ACS Med Chem Lett* 2015;6:528-530.
12. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. Systemic delivery of bone-marrow derived mesenchymal stem cells to the infracted myocardium: feasibility, cell migration and body distribution. *Circulation* 2003;108:863-868.
13. Gao J. Dennis JE, Mizic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow derived mesenchymal stem cells after infusion. *Cells Tissues Organs* 2001;169:12-20.
14. Chin BB, Nakamoto Y, Bulte JW, Pittenger MF, Wahl R, Kraitchman DL. ^{111}In oxine labelled mesenchymal stem cell SPECT after intravenous administration in myocardial infarction. *Nucl Med Commun* 2003;24:1149-1154.
15. Cui J, Wahl RL, Shen T, Fisher SJ, Recker E, Ginsburg D, Long MW. Bone marrow cell trafficking following intravenous administration. *Br J Haematol* 1999;107:895-902.
16. Allers C, Sierralta WD, Neubauer S, Rivera F, Minguell JJ, Conget PA. Dynamic of distribution of human bone marrow-derived mesenchymal stem cells after transplantation into adult unconditioned mice. *Transplantation* 2004;78:503-508.