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Development of radioiodinated lipophilic cationic compounds for myocardial imaging

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ABSTRACT

Introduction: Tc-99m compounds are mainly used in myocardial blood flow studies. These compounds, however, are produced by a generator and alternate single photon emission computed tomography (SPECT) radiopharmaceuticals are therefore required to avoid the risks posed by generator failure. Three radiolabeled compounds, including [^{125}I]*p*-iodobenzyl triphenylphosphonium ([^{125}I]ITPP), [^{125}I]*p*-Iodobenzyl dipropylphenylphosphonium ([^{125}I]IDPP) and [^{125}I]*p*-iodobenzyl methyldiphenylphosphonium ([^{125}I]IMPP), have been synthesized in the current study. All three of these compounds contain a lipophilic cation, which enhances their cell permeability properties and allows them to accumulate in the myocardium as SPECT probes.
Methods: 4-(2-Tributylstannyl) benzyl alcohol was mixed with [^{125}I]NaI in the presence of aqueous hydrogen peroxide and hydrochloric acid to allow for the synthesis of 4-[^{125}I]iodobenzyl alcohol. Bromination of the alcohol under standard conditions gave 4-[^{125}I]iodo benzyl bromide, which was treated with triphenylphosphine, dipropylphenylphosphine or methyldiphenylphosphine to give [^{125}I]ITPP, [^{125}I]IDPP and [^{125}I]IMPP, respectively. These compounds were evaluated in biodistribution and SPECT studies in normal ddY mice.
Results: All three of the radiolabeled compounds were synthesized in approximately 60% yield with radiochemical purities greater than 99%. The specific activity of each compound was 74 GBq/μmol. The results of the biodistribution and SPECT studies showed that all compounds accumulated preferentially in the heart in vivo, especially [^{125}I]IDPP.
Conclusion: [^{123}I] IDPP could be used in clinical practice as a novel myocardial imaging agent.

1. Introduction

Myocardial blood flow imaging is one of the most important imaging techniques used in nuclear medicine studies. Especially, radiopharmaceuticals labeled with Tc-99m, such as [^{99m}Tc]MIBI [1] and [^{99m}Tc]tetrofosmine [2] have been used extensively in single photon emission computed tomography (SPECT) studies because of the suitable photon energy properties of Tc-99m. Furthermore, Tc-99m can be readily accessed in reasonable quantities using a Mo-99/Tc-99m generator. The Mo-99 material used in the generator is obtained from the fission products of nuclear reactors. During the course of the last decade, there have been shortages in the supply of Mo-99 from some reactors because of maintenance issues and other problems [3,4]. This shortage in the supply of Mo-99 has led to a reduction in the amount of material available throughout the world for the production of Tc-99m. Some myocardial blood flow studies can be performed using Tl-201, but the number of studies conducted with this material has diminished during the course of the last decade. With this in mind, there is therefore an urgent need for the development of an alternate SPECT radiopharmaceutical to avoid supply issues.

The lipophilic cation-containing structures of phosphonium compounds such as tetraphenylphosphonium (TPP) are well known to be cell permeable, and compounds of this type can readily accumulate in cells as a function of the transmembrane voltage gradient [5]. The induction of the transmembrane voltage gradient is mainly dependent on the mitochondrial membrane potential (MMP) [6,7]. Cations of this type can accumulate quite readily in the mitochondria because the MMP is higher than that of any other cell organelle. [^{3}H]TPP has been widely used as an in vitro probe to measure MMP. [^{18}F]FBnTP is another radiolabeled probe, which was developed as a positron emission tomography (PET) probe by Madar et al. [8–12], following their research towards the use of [^{3}H]TPP. Notably, [^{18}F]FBnTP is currently being evaluated in clinical trials.

With this in mind, it was envisaged that phosphonium-based compounds bearing a radioiodine [^{123}I] label could be used as novel radiopharmaceuticals for myocardial imaging studies by SPECT. In this study, we have synthesized three novel compounds, including 4-[^{125}I]iodobenzyl triphenylphosphonium ([^{125}I]ITPP), 4-[^{125}I]iodobenzyl dipropylphenylphosphonium ([^{125}I]IDPP) and 4-[^{125}I]iodobenzyl methyldiphenylphosphonium ([^{125}I]IMPP), and evaluated the potential

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application of these compounds as imaging probes using biodistribution studies and SPECT images in normal mice.

2. Materials and methods

2.1. Materials

^{1}H NMR spectra were recorded at 270 MHz on a JNM-GSX-270WB spectrometer (JEOL, Tokyo, Japan) using tetramethylsilane as an internal reference. Electrospray ionization mass spectra (ESI-MS) were obtained on a Shimadzu LCMS-2020 system (SHIMADZU, Kyoto, Japan). [^{125}I]NaI was purchased from MP Biomedicals (Santa Ana, CA, USA). All of the other chemicals used in the current study were purchased as the reagent grade. All of the animals used in current study were supplied by Japan SLC (Hamamatsu, Japan). The current animal study was approved by the Animal Care and Use Committee of the Hamamatsu University School of Medicine. An automated gamma counter with a NaI (Tl) detector (Wizard™ 3; Perkin Elmer, Waltham, MA, USA) was used to measure radioactivity. SPECT/CT imaging was conducted on an FX system (Gamma Medica-Ideas, Northlidge, CA, USA).

2.2. Organic chemistry (Fig. 1)

2.2.1. Synthesis of 4-iodobenzyl triphenyl phosphonium (2a)

Triphenylphosphine (500 mg, 1.9 mmol) was added in a single portion to a solution of *p*-Iodobenzyl bromide (**1**, 500 mg, 1.68 mmol) in dry toluene (3 mL), and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was then concentrated in vacuo to give a residue, which was purified by column chromatography over silica gel eluting with a 10:1 (v/v) mixture of $CHCl_3$ and CH_3OH to yield **2a** as a white crystalline solid (951 mg, 89.5%). ^{1}H NMR ($CDCl_3$) δ: 5.55 (2H, d, J = 14.9 Hz), 6.93 (2H, dd, J = 2.16, 8.4 Hz), 7.40 (2H, d, J = 7.8 Hz), 7.74–7.82 (15H, m). ESI-MS *m/z*: 479.1 M^+, 353.2 $[M–I + H]^+$.

2.2.2. Synthesis of 4-iodobenzyl dipropyl phenyl phosphonium (2b)

Dipropylphenylphosphine (100 μL, 0.5 mmol) was added to a solution of *p*-iodobenzyl bromide (**1**, 100 mg, 0.34 mmol) in dry toluene (3 mL), and the resulting mixture was stirred at room temperature for 3 h. The solvent was subsequently removed in vacuo to give a residue, which was purified by column chromatography over silica gel eluting with a 10:1 (v/v) mixture of $CHCl_3$ and CH_3OH to yield **2b** as a white crystalline solid (35 mg, 14.3%). ^{1}H NMR ($CDCl_3$) δ: 1.09 (6H, t, J = 7.16 Hz), 1.64 (4H, m), 2.81 (4H, m), 4.71 (2H, d, J = 15.39 Hz), 6.89 (2H, d, J = 2.16, 8.37 Hz), 7.49 (2H, d, J = 7.29 Hz), 7.74 (5H, m). ESI-MS *m/z*: 411.09 M^+, 285.16 $[M–I + H]^+$.

2.2.3. Synthesis of 4-iodobenzyl methyl diphenyl phosphonium (2c)

Methyldiphenylphosphine (100 μL, 0.5 mmol) was added to a solution of *p*-iodobenzyl bromide (**1**, 100 mg, 0.34 mmol) in dry toluene (3 mL), and the resulting mixture was stirred at room temperature for 3 h. The solvent was subsequently removed in vacuo to give a residue, which was purified by column chromatography over silica gel eluting with a 10:1 (v/v) mixture of $CHCl_3$ and CH_3OH to yield **2c** as a white crystalline solid (35 mg, 14.1%). ^{1}H NMR ($CDCl_3$) δ: 2.67 (3H, d, J = 13.77 Hz), 4.95 (2H, d, J = 15.66 Hz), 7.04 (2H, d, J = 8.1 Hz), 7.45 (2H, d, J = 7.83 Hz), 7.752 (10H, m). ESI-MS *m/z*: 417.05 M^+, 291.15 $[M–I + H]^+$.

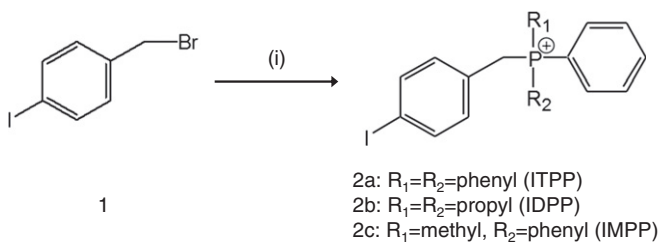

Fig. 1. Synthesis of p-iodobenzyl phosphonium cations. Reagents and conditions: (i) triphenylphosphine (**2a**), dipropylphenylphosphine (**2b**), methyldiphenylphosphine (**2c**), in dry toluene, stirred at r.t. for 3 h.

2.3. Radiochemistry (Fig. 2)

2.3.1. Synthesis of 4-(2-tributylstannyl) benzyl alcohol (4)

A mixture of *p*-iodobenzyl alcohol (**3**, 50 mg, 0.21 mmol), bis(tributyltin) (372 mg, 0.64 mmol) and tetrakis(triphenylphosphine)palladium (16 mg, 0.014 mmol) in dry toluene (5 mL) was heated at refluxed under an atmosphere of argon for 3 h. The mixture was then cooled to ambient temperature and filtered through celite. The filtrate was concentrated in vacuo to give an oily residue, which was dissolved in ethyl acetate and washed sequentially with 5% (v/v) aqueous hydrochloric acid (HCl) and a saturated aqueous solution of $NaHCO_3$ before being dried over Na_2SO_4. The solvent was then removed in vacuo to give an oily residue, which was purified by column chromatography over silica gel eluting with $CHCl_3$ to yield **4** as colorless oil (18 mg, 30.4%). ^{1}H NMR ($CDCl_3$) δ: 0.86–1.70 (27H, m, Bu_3), 4.67 (2H, d, J = 5.9 Hz), 7.33 (2H, d, J = 7.8 Hz), 7.47 (2H, d, J = 7.8 Hz),

2.3.2. Radiolabeling of 4-[^{125}I]iodobenzyl alcohol (5)

4-[^{125}I]Iodobenzyl alcohol (**5**) was radiolabeled according to a previously reported method [13]. Briefly, aqueous hydrogen peroxide (10 μL, 30% w/v) was added to a mixture of [^{125}I]NaI (10 μL, 37.0 MBq, 74 GBq/μmol), 0.1 N HCl (100 μL) and tributylstannyl precursor **4** (0.01 mg in 10 μL of ethanol) in a sealed vial, and the resulting mixture was stirred for 30 min at room temperature. The reaction was then quenched by the addition of aqueous sodium bisulfite (0.1 mg in 10 μL), and the resulting mixture was purified by HPLC over a COSMOSIL 5C_{18}-AR-II column (10 × 250 mm) eluting with a 1:9 (v/v) mixture of 5 mM CH_3COONH_4 buffer (pH 4) and CH_3OH at a flow rate of 3.0 mL/min. Fraction containing 4-[^{125}I]iodobenzyl alcohol (**5**) were combined and evaporated to dryness in vacuo.

2.3.3. Synthesis of 4-[^{125}I]iodobenzyl triphenylphosphonium (7a), 4-[^{125}I]iodobenzyl dipropylphenylphosphonium (7b) and 4-[^{125}I]iodobenzyl methyldiphenylphosphonium (7c)

Phosphorus tribromide (50 μL, 0.5 mmol) was added to a solution of **5** in diethyl ether (1 mL), and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was quenched by the addition of H_2O, and the resulting mixture was extracted with diethyl ether to give 4-[^{125}I]Iodobenzyl bromide (**6**). Triphenylphosphine (10 mg, 38 μmol), dipropylphenylphosphine (5 μL, 25 μmol) or methyldiphenylphosphine (5 μL, 25 μmol) was then added to the diethyl ether solution of 4-[^{125}I]iodobenzyl bromide (**6**), and the resulting mixture was stirred for 30 min at 40 °C in a sealed vial. The mixture was then cooled to room temperature and the solvent was evaporated under a gentle stream of Ar gas. The resulting residue was dissolved in CH_3OH and purified by HPLC eluting with 1:9 (v/v) mixture of 5 mM CH_3COONH_4 buffer (pH 4) and CH_3OH at a flow rate of 3.0 mL/min to give **7a**, **7b** or **7c**. The radiolabeling experiments were carried out under carrier-free conditions.

2.4. Estimation of the lipophilicity

The distribution coefficient (D) values of the compounds between *n*-octanol and buffer were measured using a standard shake-flask method according to a previously reported method [14]. Briefly, the radioactive sample (1 μL) was shaken in a mixture of *n*-octanol (1 mL) and 66 mM phosphate buffer (pH 7.4, 1 mL) for 5 min at room temperature. The biphasic mixture was then centrifuged at 2500×*g* for 5 min and the