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Radiosynthesis and preliminary biological evaluation of a new ^{18}F-labeled triethylene glycol derivative of triphenylphosphonium†

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Delocalized lipophilic cations such as [^{18}F]fluorobenzyltriphenylphosphonium ([^{18}F]FBnTP) can accumulate in mitochondria and have been used in myocardial perfusion imaging (MPI). In this study, we established a simplified method for [^{18}F]FBnTP synthesis using triphenylphosphine hydrobromide (PPh_3•HBr) without preparing an intermediate that contains benzyl bromide structure. Applying this new method, we synthesized and evaluated a novel ^{18}F-labeled PEGylated BnTP derivative ([^{18}F]FPEGBnTP). *In vitro* cellular uptake study demonstrated that [^{18}F]FPEGBnTP accumulated in cells in proportion to the relative intensity of mitochondrial membrane potential. Biodistribution study revealed that the heart : liver uptake ratio of [^{18}F]FPEGBnTP (4.00 at 60 min) was superior to that of [^{18}F]FBnTP (1.50 at 60 min). However, [^{18}F]FPEGBnTP showed slow blood clearance and high radioactivity uptake in bone at 120-min post-injection. These results imply the possibility of [^{18}F]FPEGBnTP being used as a MPI agent. However, there is a need of further structural optimization and flow-dependent uptake study.

Keywords: mitochondria; FBnTP; DLCs; positron emission tomography; PET

Introduction

According to a report from the World Health Organization, the leading cause of death worldwide is ischemic heart disease, and 7.4 million people died in 2012.[1] Early and accurate detection of cardiac ischemia enables appropriate therapy to be instigated and reduces the risk of disease progression.

Myocardial perfusion imaging (MPI) is a tool for non-invasive evaluation of cardiac ischemia. Tracers based on technetium-99m (^{99m}Tc), such as [^{99m}Tc]sestamibi and [^{99m}Tc]tetrofosmin, are used as MPI agents for single-photon emission computed tomography (SPECT).[2] With regard to the mechanism of accumulation of these ^{99m}Tc-labeled tracers, the association of mitochondrial membrane potentials (MMPs) in myocardial cells has been considered.[2,3] It has been suggested that these tracers remain in the heart for a long time and that tracers based on thallium-201 are not redistributed.[3] However, these tracers also accumulate in organs adjacent to the heart and interfere with diagnostic imaging.[3,4] Compared with SPECT, positron emission tomography (PET) has technical advantages: special resolution, attenuation correction, sensitivity, and quantitation.[5] PET tracers such as [^{13}N]NH_3 and [^{15}O]H_2O are used for MPI,[6,7] but application of these tracers is limited to institutions with on-site cyclotrons because of their short half-lives ($T^{1/2}$ = 9.97 min and 122 s, respectively). Conversely, PET tracers labeled with fluorine-18 (^{18}F) have a suitable half-life ($T^{1/2}$ = 109.8 min), which enables their delivery to many PET centers and has contributed to their increased use. Accordingly, drug design involving ^{18}F for MPI has been undertaken.

Use of [^{18}F]fluorobenzyltriphenylphosphonium ([^{18}F]FBnTP) was reported first in 2004.[8] This tracer accumulates in the myocardium to the same extent as [^{99m}Tc]sestamibi and [^{99m}Tc]tetrofosmin. [^{18}F]FBnTP has been used as a 'gold standard' PET tracer for MPI,[9–11] but its performance can be improved. This tracer accumulates in the liver to the same extent as that seen in the heart and exhibits slow clearance from the liver, which can adversely affect MPI. To optimize pharmacokinetics, several research teams have developed new phosphonium derivatives.[12,13]

This study aims to develop a new BnTP derivative showing lower liver uptake and higher heart-to-liver ratio in comparison with [^{18}F]FBnTP. We hypothesized that the lipophilicity of [^{18}F]FBnTP would affect its accumulation in the liver and a lower lipophilic derivative would indicate faster clearance from the liver. To reduce the lipophilicity, we designed and synthesized a ^{18}F-labeled triethylene glycol derivative of BnTP ([^{18}F]FPEGBnTP). In the synthesis, we established a novel synthetic route using triphenylphosphine hydrobromide (PPh_3 • HBr) without preparing an intermediate that contains benzyl bromide structure. Then,

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we preliminarily evaluated *in vitro* cellular uptake and *in vivo* biodistribution of [^{18}F]FPEGBnTP to elucidate the possibility for an MPI agent.

Materials and methods

Synthesis of chemical compounds

Methods for the synthesis and characterization data of chemical compounds (shown in Schemes 1–4) are noted in detail in Supporting Information.

Measurement of octanol/water partition coefficient

As an index for lipophilicity, octanol/water partition coefficient (LogP) values of FBnTP and FPEGBnTP were determined by conventional shake flask method using n-octanol saturated with water and water saturated with n-octanol. Briefly, an octanol solution of FBnTP or FPEGBnTP (1 mL, 1 mg/mL) was mixed with octanol (2 mL) and water (3 mL) in a conical tube. After shaking for 30 min, the mixture was centrifuged to separate the octanol–water phases (500 rpm, 20 min). Relative amount of test compound in each phase was determined by analytical reverse-phase high-performance liquid chromatography (RP-HPLC; column: Inertsil® ODS-4 (4.6 × 150 mm); mobile phase: acetonitrile/1× phosphate-buffered saline (PBS; 50/50); flow rate: 1.5 mL/min; UV: 254 nm).

Radiochemistry

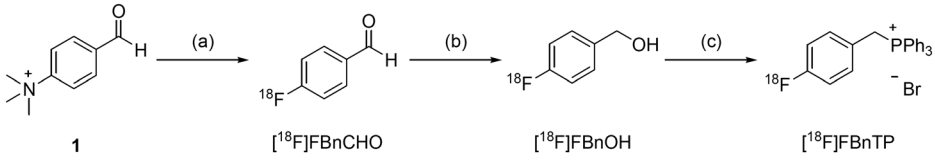
No carrier-added ^{18}F was produced by the ^{18}O(p, n)^{18}F reaction on enriched [^{18}O]H_2O (Taiyo Nippon Sanso, Tokyo, Japan) with an HM-12 cyclotron (Sumitomo Heavy Industries, Tokyo, Japan) installed in the Cyclotron and Radioisotope Center of Tohoku University (Sendai, Japan). The specific activity of ^{18}F was 74–740 GBq/mmol at the end of bombardment. Radiochemical purity was determined by radioanalytical HPLC under the same HPLC conditions used for LogP measurement.

Radiosynthesis of [^{18}F]FBnTP

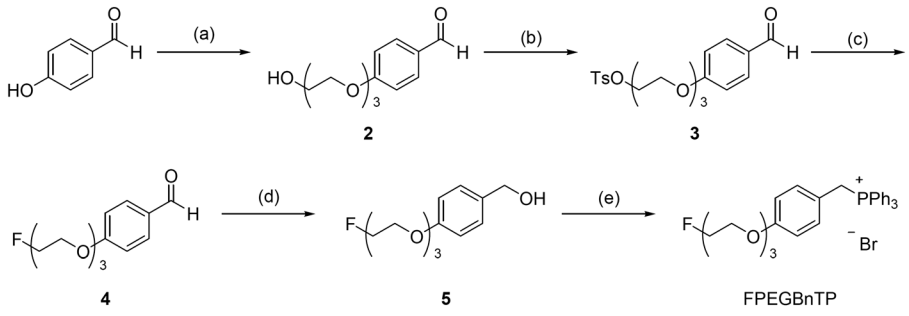
The aqueous [^{18}F]F^- contained in K_2CO_3 solution (3.7–5.6 GBq) and Kryptofix 2.2.2 (16 mg) were placed in a brown vial. Then, water was removed by azeotropic means with acetonitrile by heating at 110 °C with helium gas flow. After drying, precursor **1** (15 mg) in dimethyl sulfoxide (DMSO) (0.6 mL) was added and heated at 150 °C for 10 min, followed by the addition of water for quenching. The product was extracted by solid-phase extraction (SPE) with a Sep-Pak tC18 Plus Long Cartridge (Waters, Milford, MA, USA). Then, an aqueous solution of $NaBH_4$ (20 mg/mL) was added to the cartridge to reduce the aldehyde to an alcohol. The cartridge was washed with water and the water drained off. Then, the product was eluted with *o*-xylene (4.1 mL). The eluent was dried by passing through an EXtrelut® NT1 column (Merck, Whitehouse Station, NJ, USA). The anhydrous [^{18}F]BnOH solution eluted from the EXtrelut NT1 column was added to a brown vial containing PPh_3 • HBr (126 mg). The reaction mixture was heated at 150 °C for 10 min, followed by addition of $CHCl_3$ (6 mL). The product was extracted by SPE with a Sep-Pak tC18 Plus Short Cartridge (Waters). The cartridge was washed with $CHCl_3$ (5 mL) and Et_2O (5 mL). The product was eluted with ethanol. The ^{18}F-labeled product was purified from the eluent by semi-preparative RP-HPLC (column: Inertsil® ODS-4 (10 × 250 mm); mobile phase: acetonitrile/1× PBS (52/48); flow rate: 5.0 mL/min; UV: 254 nm). The ^{18}F-labeled product was isolated from the collected fraction by SPE with a Sep-Pak tC18 Plus Short Cartridge (Waters) and finally dissolved in saline for biologic evaluation. At HPLC, the radiochemical yield was 12–14% ($n = 5$). Radiochemical purity of the drug solution was >99% (Figure 3a).

Radiosynthesis of [^{18}F]FPEGBnTP

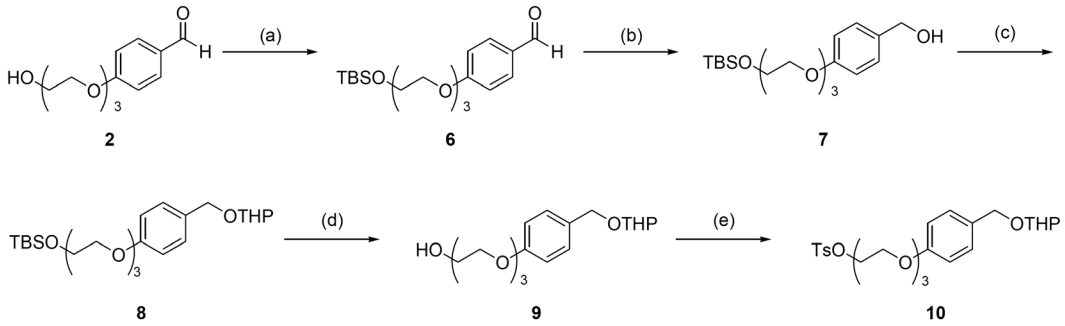
The aqueous [^{18}F]F^- contained in K_2CO_3 solution (3.7–5.6 GBq) and Kryptofix 2.2.2 (16 mg) were placed in a brown vial. Then, water was removed by azeotropic means with acetonitrile by heating at 110 °C and helium gas flow. After drying, precursor **10** (3 mg) in DMSO (0.6 mL) was added and heated at 110 °C for 10 min. Then, 0.5 M HCl was added to the solution and heated for an additional 3 min. After neutralization with 1 M AcOK, the product was extracted by SPE with a Sep-Pak tC18 Plus Short Cartridge (Waters). Then, the product was eluted with *o*-xylene (4.1 mL). The eluent was dried by passing through an EXtrelut NT1 column (Merck). The anhydrous solution eluted from the



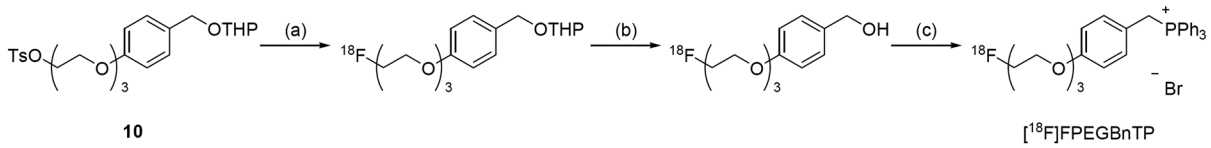
Scheme 1. Synthesis of [^{18}F]FBnTP. Reaction conditions: (a) [^{18}F]KF, K.2.2.2, DMSO, 150 °C, 10 min; (b) $NaBH_4$aq on a Sep-Pak tC18 Plus Short Cartridge; and (c) PPh_3 • HBr, *o*-xylene, reflux, 10 min.



Scheme 2. Synthesis of FPEGBnTP. Reaction conditions: (a) 2-[2-(2-Chloro-ethoxy)-ethoxy]-ethanol, K_2CO_3, DMF, 100 °C, 15 h; (b) *p*-TsCl, NMM, DCM, r.t., 2 h; (c) TBAF, THF, reflux, 2 h; (d) $NaBH_4$, MeOH, r.t., 2 h; and (e) PPh_3·HBr, MeCN, reflux, 11 h.



Scheme 3. Synthesis of precursor of FPEGBnTP. Reaction conditions: (a) TBS-Cl, DMAP, DMF, 100 °C, 15 h; (b) $NaBH_4$, MeOH, 0 °C, 30 min; (c) 2,3-DHP, PPTs, DCM, r.t., 1 h; (d) TBAF, THF, r.t., 15 min; and (e) *p*-TsCl, NMM, DCM, r.t., 11 h.



Scheme 4. Synthesis of [^{18}F]FPEGBnTP. Reaction conditions: (a) [^{18}F]KF, K.2.2.2, DMSO, 110 °C, 10 min; (b) HClaq, 110 °C, 3 min; and (c) PPh_3·HBr, *o*-xylene, reflux, 10 min.

column was added to a brown vial containing PPh_3•HBr (126 mg). The reaction mixture was heated at 150 °C for 10 min, followed by addition of $CHCl_3$ (6 mL). The product was extracted by SPE with a Sep-Pak tC18 Plus Short Cartridge (Waters). The cartridge was washed with $CHCl_3$ (5 mL) and Et_2O (5 mL). The product was eluted with ethanol. The ^{18}F-labeled product was purified from the eluent by semi-preparative RP-HPLC (column: Inertsil® ODS-4 (10× 250 mm); mobile phase: acetonitrile/1× PBS (52/48); flow rate: 5.0 mL/min; UV: 254 nm). The ^{18}F-labeled product was isolated from the collected fraction by SPE with a Sep-Pak tC18 Plus Short Cartridge and finally dissolved in saline for biologic evaluation. At HPLC, the radiochemical yield was 13–23% ($n = 8$). Radiochemical purity of the drug solution was >95% (Figure 3b).

Cellular uptake

Four types of cells were used: human cholangiocarcinoma cells (TFK1) and human breast cancer cells (MCF7, MB231, MB453). These cells were cultured with RPMI-1640 medium and used for uptake studies (3.3×10^6 cells/mL of medium).

A cell line was suspended in 150 μL of serum-free RPMI-1640 medium (TFK1, MCF7, MB231, MB453) and added to a 96-well plate (5.0×10^5 cells/well, $n = 8$). [^{18}F]FPEGBnTP in PBS (50 μL; 1.85 MBq/mL at the start of experiments; radiochemical purity >99%) was added to each well and incubated at 37 °C for 30 min. Then, cells were harvested through suction filtration with a vacuum manifold (Millipore, Bedford, MA, USA). Trapped cells were washed three times with PBS (200 mL/well). Filters holding the trapped cells were separated, and radioactivity was measured with a γ-counter (AccuFLEX g7000; Hitachi Aloka Medical, Tokyo, Japan). Then, percentage of the radioactivity of the cell to total radioactivity of a solution in a blank well (containing media but no cell) was calculated as an index of cellular uptake.

Assessment of relative intensity of mitochondrial membrane potential

Correlation between the relative intensity of mitochondrial membrane potential (MMP) and cellular uptake of [^{18}F]FPEGBnTP was evaluated using a JC-10 Mitochondrial Membrane Potential Assay kit (Microplate; Abcam, Cambridge, UK) and four human cancer cell lines (TFK1, MCF7, MB231, and MB453). Cells in 90 μL of RPMI-1640 medium were added to a 96-well plate (5.0×10^5 cells/well, $n = 8$). The medium not containing cells was added as a control. Then, 10 μL of PBS was added to each well and incubated at 37 °C for 30 min. Fifty microliters of JC-10 solution diluted with Assay Buffer A was added to each well and incubated at 37 °C for 30 min. Then, 50 μL of Assay Buffer B was added to each well and fluorescence intensity read with a microplate reader. Excitation was at 495 nm; emission was read at 520 and 590 nm. The ratio of Em_{590} to Em_{520} was calculated. The percentage of the control value was used as an index of the relative intensity of MMP.

Biodistribution assay in normal mice

Study protocols using animals were approved by the Institutional Animal Care and Use Committee of the Tohoku University Environmental & Safety Committee. Male ICR mice (25–35 g) were injected in a lateral tail vein with ^{18}F-labeled tracer (370 ± 30 kBq) in saline (0.2 mL). Mice were killed by cervical dislocation after heart puncture to obtain blood samples at designated time points post-injection ($n = 4$ at 60 and 120 min). Tissues of interest were excised and weighed, and the radioactivity was counted in an automatic gamma counter. Data relating to radioactivity uptake are expressed as percent injected dose per gram of tissue (%ID/g).

Results

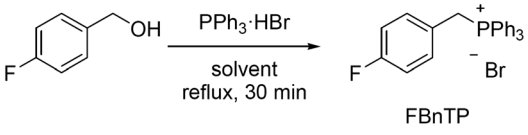
Radiosynthesis of [^{18}F]FBnTP

Total synthesis of [^{18}F]FBnTP was planned as shown in Scheme 1. First, the solvent condition of the phosphonium formation step was examined in the non-radioactive condition (Figure 1). The reaction was carried out so that FBnOH (9.16 μmol) and excess PPh_3•HBr (400 μmol) in various solvents were heated at reflux for 30 min. Results revealed that *o*-xylene (non-polar solvent with a high boiling point) gave the best yield. We estimated that using *o*-xylene would also give the best radiochemical yield in the radioactive condition.

Then, we worked on the first nucleophilic aromatic substitution step in Scheme 1. Compound **1** was prepared by a slight modification of a procedure reported previously.[14] After the reaction, the crude product, [^{18}F]FBnCHO, was extracted by SPE with a Sep-Pak tC18 Plus Long Cartridge (Waters). Subsequently, an aqueous solution of $NaBH_4$ was added to the cartridge to reduce the aldehyde to an alcohol. The crude product containing [^{18}F]FBnOH was eluted with *o*-xylene. HPLC analyses revealed that the reduction reaction proceeded quantitatively. With respect to the two steps so far, various reaction conditions were tested. When dimethylformamide was used as a solvent at 150 °C, it gave [^{18}F]FBnOH with the best radiochemical yield.

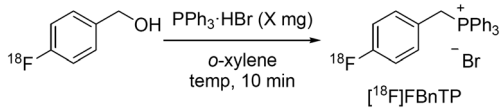
At the next phosphonium formation step using excess PPh_3•HBr, the reaction did not proceed at all. A possible cause was the water contained in the reaction mixture. Hence, after drying the mixture with magnesium sulfate, the reaction was tried again and [^{18}F]FBnTP was obtained. To simplify this dehydration step, various commercially available column cartridges were tested. Among them, EXtrelut NT1 (Merck) could be used to dehydrate the reaction mixture to give [^{18}F]FBnTP.

Finally, we examined the amount of PPh_3•HBr and the reaction temperature under radioactive conditions. At first, a small amount of PPh_3•HBr was applied to the reaction, but [^{18}F]FBnTP was not detected (entries 1–3, Figure 2). Hence, the amount of PPh_3•HBr and reaction temperature were increased (entries 4–7, Figure 2). [^{18}F]FBnTP could be obtained at the best yield when 126 mg of PPh_3•HBr was used. Under this condition, a large amount of unreacted PPh_3 remained in the reaction mixture. Fortunately, the retention value of FBnTP is much lower than that of PPh_3, so [^{18}F]FBnTP could be obtained after PPh_3 was first removed. Hence, the reaction mixture was placed on a Sep-Pak tC18 Plus Short Cartridge and washed with $CHCl_3$ and Et_2O followed by extraction of [^{18}F]FBnTP with MeOH. [^{18}F]FBnTP was purified further with semi-preparative RP-HPLC using an acetonitrile/1× PBS phase. After the appropriate fraction was collected, analytical HPLC was carried out and showed a single product peak, which suggested that [^{18}F]FBnTP was obtained at high purity. Radiochemical yield was 12–14% ($n = 5$, decay-corrected). Radiochemical purity was >99%.



entry	solvent	yield (%)[a]
1	*o*-xylene	54
2	toluene	36
3	$CHCl_3$	5.2
4	CH_2Cl_2	0.43
5	DMSO	-[b]
6	DMF	-
7	MeCN	-

Figure 1. Solvent condition of phosphonium formation. Superscript lowercase a indicates that yield was measured by HPLC; superscript lowercase b indicates that yield was not determined because of the product peak overlapping the peak for by-products.



entry	X (mg)	temp. (°C)	R.Y.[a] (%)
1	2.5	120	-
2	10	120	-
3	20	120	-
4	36	140	27
5	63	140	49
6	63	150	33
7	126	150	56

Figure 2. Synthesis of [^{18}F]FBnTP. Reaction conditions of phosphonium formation. R.Y., radiochemical yield (decay-corrected) measured by HPLC.

Synthesis of FPEGBnTP and its precursor

The reference compound FPEGBnTP was prepared in the five steps shown in Scheme 2. The precursor was also synthesized according to Scheme 3 by five steps from 2. These compounds were characterized by infrared, ^{1}H-NMR, ^{13}C-NMR, and high-resolution mass spectrometry or fast atom bombardment mass spectrometry.

Measurement of octanol/water partition coefficient

To compare the lipophilicity between FBnTP and FPEGBnTP, octanol/water partition coefficient (LogP) of them were measured. LogP values of FBnTP and FPEGBnTP were −0.38 and −0.92, respectively, indicating that FPEGBnTP is relatively less lipophilic than FBnTP.

Radiosynthesis of [^{18}F]FPEGBnTP

The radiosynthetic route was planned and undertaken as shown in Scheme 4. Briefly, precursor **10** was reacted with the [^{18}F]F^- activated with Kryptofix 2.2.2. An aqueous solution of HCl was added to deprotect the hydroxyl group. At the next phosphonium formation step, our method using PPh_3•HBr was applied. After purification with RP-HPLC, analytical HPLC was undertaken and showed a single product peak. Radiochemical yield was 13–23% ($n = 8$, decay-corrected). Radiochemical purity was >95%.

Cellular uptake

The relative intensity of MMP was assayed with a JC-10 that is a kind of fluorescent delocalized lipophilic cation (DLC) and accumulates in mitochondria. JC-10 has usually maximal fluorescence at 520 nm with an excitation wavelength at 495 nm. However, when the concentration of JC-10 become high in mitochondria, an aggregate of JC-10 is formed and the emission peak shifts to 590 nm. The relative intensity of MMP can be evaluated by measuring the ratio of fluorescence intensity between at 520 and 590 nm. The relative intensity of MMP was expressed as percent Em_{590}/Em_{520} of the control and is summarized in Table 1. Results of percent applied activity of [^{18}F]FPEGBnTP uptakes were summarized in Table 2. These data are plotted on a scatter diagram and showed a linear correlation between the relative intensity of MMP and cellular uptake of [^{18}F]FPEGBnTP (Figure 3).

Biodistribution study in normal mice

Properties of [^{18}F]FPEGBnTP were evaluated by a biodistribution study in normal mice ($n = 4$ at each time point). Radioactivity

Table 1. Cellular uptake. JC-10 assay for mitochondrial membrane potential

Cellular	% Ratio (Em590/Em520) of control
TFK1	154 ± 2
MB231	150 ± 1
MB453	130 ± 5
MCF7	124 ± 2
Control	100 ± 2

Values of percent ratio (Em_{590}/Em_{520}) of the control are mean ± SD ($n = 8$).

Table 2. Cellular uptake of [^{18}F]FPEGBnTP

Cellular	% Applied activity
TFK1	1.96 ± 0.16
MB231	1.76 ± 0.10
MB453	0.78 ± 0.17
MCF7	0.09 ± 0.07

Values of percent applied activity are mean ± SD ($n = 8$).

uptake in the blood, heart, and other organs are summarized in Table 3. With regard to heart uptake, each time point showed adequate uptake (3.33%ID/g at 60 min). By contrast, the neighboring organs such as liver and lung exhibited lower uptakes (0.83 and 0.81%ID/g at 60 min, respectively), resulting in higher ratios of heart : liver and heart : lung. However, radioactivity uptakes in blood were relatively higher than those of liver and lung. Kidney showed the highest radioactivity uptake at 60 min post-injection. The bone uptake increased with elapsed time from 60 to 120 min post-injection.

Discussion

Accumulation of [^{18}F]FBnTP in the heart is because of abundant mitochondria in myocardial cells. [^{18}F]FBnTP is a DLC. DLCs are small molecules that carry a positive charge. Their chemical structures allow the charge to be 'delocalized' over a large molecular area by resonance stabilization. The lipophilicity of DLCs enables them to cross the lipid bilayers of cells and to access the internal environments of mitochondria.[15] At the inner membranes of mitochondria, a pH gradient is formed by electron transport systems, so a strongly negative membrane potential develops. After passing through the lipid bilayers of cells, DLCs accumulate preferentially in the inner membranes of mitochondria driven by the MMP.

[^{18}F]FBnTP is phosphonium salt DLC that shows promising characteristics as a ^{18}F-labeled MPI tracer.[11–13] Additionally, this tracer has been used for studies on imaging of brown adipose tissue and apoptosis.[16–18] In a synthetic route for [^{18}F]FBnTP reported in 2004,[10] a corrosive reagent, PPh_3Br_2, was used in the step for [^{18}F]FBnBr synthesis. This reagent is not suitable for automatic synthetic apparatus or conventional use.

We proposed a new synthetic route using PPh_3•HBr, which is not corrosive (Scheme 1). Our route could lead to [^{18}F]FBnTP from [^{18}F]FBnOH in one step. However, from the result of the phosphonium formation step, excess PPh_3•HBr turned out to be necessary (Figure 2). We suspected that if a small amount of PPh_3•HBr was used, the hydroxyl group of [^{18}F]FBnOH would not be activated adequately, and the reaction would not proceed completely. After this reaction, we were able to isolate [^{18}F]FBnTP from excess PPh_3 on a Sep-Pak tC18 Plus Short Cartridge. At the HPLC separation step, [^{18}F]FBnTP could be obtained within a reasonable range and not be affected by PPh_3. Radiochemical yield was 12–14% ($n = 5$, decay-corrected), in accordance with that obtained by the conventional method. Our new method is thought to be practical and environmentally friendly. Nevertheless, a complicated dehydration procedure is necessary after the reduction step. There is room for further improvement that does not require dehydration, such as using a $NaBH_4$-containing alumina column.

There was a problem regarding the pharmacokinetics of [^{18}F]FBnTP: This tracer accumulated in the liver. The liver is adjacent to the heart, which could adversely affect MPI. To improve the pharmacokinetics of [^{18}F]FBnTP, we designed FPEGBnTP, which increased hydrophilicity by introduction of a polyethylene glycol (PEG) chain. In the synthesis of [^{18}F]FPEGBnTP, our phosphonium formation method with PPh_3•HBr could be applied and produce a good yield (13–23%, $n = 8$, decay-corrected).

Table 3. Biodistribution of [^{18}F]FPEGBnTP at 60 and 120 min after intravenous injection

	%ID/g		Heart : tissue ratio	
	60 min	120 min	60 min	120 min
Heart	3.3 ± 0.22	3.61 ± 0.26		
Blood	1.08 ± 0.05	1.00 ± 0.05	3.13 ± 0.35	3.61 ± 0.02
Liver	0.83 ± 0.02	0.81 ± 0.04	4.00 ± 0.28	4.46 ± 0.02
Lung	0.81 ± 0.01	0.95 ± 0.04	4.10 ± 0.37	3.80 ± 0.06
Spleen	0.89 ± 0.04	0.91 ± 0.06	3.74 ± 0.27	3.97 ± 0.06
Kidney	3.64 ± 0.18	1.08 ± 0.05	0.92 ± 0.09	3.34 ± 0.03
Small intestine	1.94 ± 0.10	1.54 ± 0.18	1.74 ± 0.19	2.34 ± 0.08
Muscle	1.28 ± 0.06	1.38 ± 0.13	2.60 ± 0.20	2.62 ± 0.07
Bone	2.11 ± 0.08	3.88 ± 0.29	1.58 ± 0.14	0.93 ± 0.02
Brain	0.69 ± 0.02	0.73 ± 0.04	4.81 ± 0.31	4.95 ± 0.02

Values of %ID/g and heart : tissue ratio are mean ± SD for four mice.

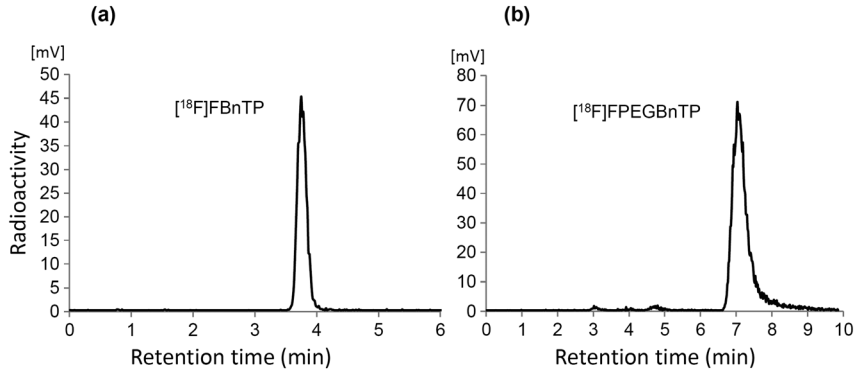


Figure 3. Radioanalytical HPLC chromatograms. (a) [^{18}F]FBnTP and (b) [^{18}F]FPEGBnTP.

Biological features of [^{18}F]FPEGBnTP were evaluated by *in vitro* cellular uptake assay and *ex vivo* biodistribution assay in mice. Using four cancer lines, the relationship between the relative intensity of MMP and cellular uptake of [^{18}F]FPEGBnTP was examined. Generally, MB231 cells show significant reduction in mitochondrial functions including electron transport chain activities, oxygen consumption, and ATP synthesis rates.[19] These features would raise an expectation that MMP of MB231 cells is lower than that of MCF7 cells. However, our study using a fluorescent dye JC-10 for MMP measurement resulted in reversed relationship between MB231 and MCF7 cells. We speculate that if the total amount of mitochondria included in 5.0×10^5 cells of MB231 is greater than that of MCF7, it would be possible to be greater fluorescent signal of MB231 than MCF7. Similar to our results, Cheng et al. reported that JC-1, an analogue of JC-10, signal ratio (% of control) of MB231 cells was greater than that of MCF7.[20] However, a result contrary to that was also reported.[21] The importance of the cellular uptake assay is to reveal the correlation between cellular MMP and uptakes. As shown in Figure 4, [^{18}F]FPEGBnTP accumulated in cells in proportion to the relative intensity of MMP, suggesting that this tracer shows an MMP-dependent cellular uptake *in vitro*.

In vivo behavior of [^{18}F]FPEGBnTP was investigated by a biodistribution assay. The heart : liver ratio of [^{18}F]FPEGBnTP (4.00 at 60 min) was superior to that of [^{18}F]FBnTP (1.50 at 60 min) reported by Madar et al.,[22] indicating that the liver pharmacokinetics of [^{18}F]FPEGBnTP was improved. Conversely, the heart : blood ratio of [^{18}F]FPEGBnTP was low compared with that of [^{18}F]FBnTP,[22] suggesting that the clearance of [^{18}F]FPEGBnTP from blood was slower as compared with [^{18}F]FBnTP. The slow blood clearance of the activity might be caused by non-specific binding of [^{18}F]FPEGBnTP or its metabolites to the plasma constituents because the activity level showed little change from 60 to 120 min after injection. In addition, increasing accumulation of radioactivity in bone suggests a low tolerance to defluorination of [^{18}F]FPEGBnTP *in vivo*. There is a possibility that these metabolism natures of [^{18}F]FPEGBnTP have caused the prolonged recirculation of activity of metabolites in the vascular system and/or the relatively lower heart uptake. Hence, although the high heart : liver ratio of [^{18}F]FPEGBnTP implies the possibility for using it as a MPI agent, there is a need of a detailed metabolism study, flow-dependent uptake in heart, and further chemical modification to show better *in vivo* stability leading to more favorable pharmacokinetics.

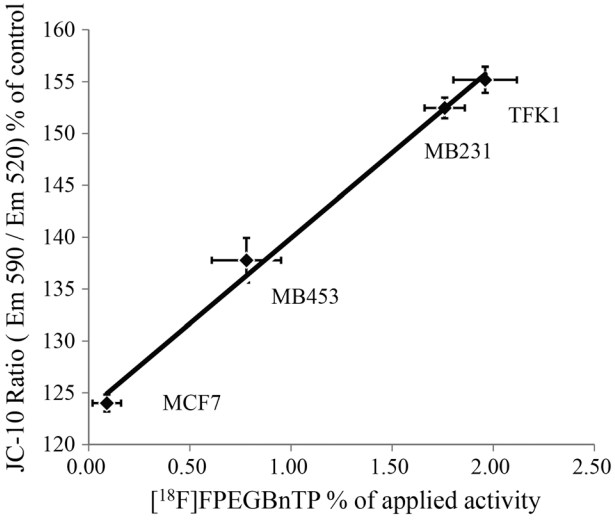


Figure 4. Correlation of cellular uptake of JC-10 and [^{18}F]FPEGBnTP. A linear correlation was observed ($R^2 = 0.9972$).

Conclusion

We developed a new PEGylated phosphonium derivative [^{18}F]FPEGBnTP, which accumulated in a particular cancer line in proportion to the MMP. Biodistribution study demonstrated the high uptake in the heart and good heart : liver and heart : lung ratios, suggesting that [^{18}F]FPEGBnTP would be a possible candidate for a new ^{18}F-labeled MPI agent. However, there is still room for optimization of the radiosynthesis procedures and the chemical structure and is a need of studies on metabolism and flow-dependent uptake in the heart to prove the usefulness for using as a MPI agent.

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