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Effect of methoxy group position on biological properties of ^{18}F–labeled benzyl triphenylphosphonium cations

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ABSTRACT

Introduction: ^{18}F–labeled phosphonium cations targeting mitochondrial membrane potential would be promising for positron emission tomography (PET) myocardial perfusion imaging (MPI). The purpose of this study was to examine the influence of additional methoxy group and its different positions on myocardium uptake and pharmacokinetics properties of ^{18}F–labeled benzyl triphenylphosphonium cations.
Method: In this study, three novel ^{18}F–labeled phosphonium cations, [^{18}F]4-(fluoromethyl)benzyltris(4-methoxyphenyl) phosphonium cation (**1b**), [^{18}F]4-(fluoromethyl)benzyltris(2-methoxyphenyl) phosphonium cation (**2b**) and [^{18}F]4-(fluoromethyl)benzyltris(3-methoxyphenyl) phosphonium cation (**3b**), were efficiently prepared by a One-Pot method starting from the substitution of non-carried-added fluoride-18. Radiotracers were purified by HPLC. Physicochemical properties, *in vitro* cell uptake assay, *in vivo* mice biodistribution and rat micro-PET imaging were investigated.
Results: Results suggested that the position of methoxy group exhibited significant effect on the biological properties of ^{18}F–labeled benzyl triphenylphosphonium cations. The addition of methoxy group on *orth-* or *meta-* position of the radiotracers accelerated the radioactivity clearance from liver. The *para*-radiotracer had the highest uptake in the heart and other non-targeting organs. According to the biodistribution data, **2b** (*ortho-*) displayed the fastest liver clearance and highest heart-to-background ratios. And its rat micro-PET images at 60 min post-injection revealed a good visualization of heart and favorable heart-to-background contrast. Nevertheless, **2b** exhibited a lower initial liver uptake and quicker liver clearance compared with ^{99m}Tc-sestamibi.
Conclusion: The *ortho-* compound (**2b**) displayed the most favorable biological properties as a potential MPI agent to acquire high contrast images early after injection.

1. Introduction

In the current clinical nuclear diagnostic technologies, single photon emission computed tomography (SPECT) with the ^{99m}Tc-complexes, such as ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin, is the mainstay of myocardial perfusion imaging (MPI) [1–3]. Compared to SPECT, positron emission tomography (PET) with positron emitting tracers such as ^{15}O–water, ^{13}N–ammonia and ^{82}Rubidium has advantages in terms of higher spatial resolution, availability of attenuation correction and quantitation of myocardial blood flow (MBF) [4–7]. But the short half-life of the available nuclides, such as ^{15}O ($t_{1/2}$ = 2 min), ^{13}N ($t_{1/2}$ = 10 min), and ^{82}Rb ($t_{1/2}$ = 75 s), limits their clinical use [8,9]. Compared to the short half-life nuclides, ^{18}F has a more widespread clinical application because of the relative longer half-life for about 109 min and fairly appropriate positron range. Therefore, development of novel ^{18}F–labeled MPI agents has been a focus of interest in the radiopharmaceutical field [10,11].

Similar to ^{99m}Tc-sestamibi, lipophilic phosphonium cations can accumulate in cardiomyocytes as potential MPI agents, due to the higher mitochondrial membrane potential in cardiomyocytes [12–17]. Over the past few years, two major types of ^{18}F–labeled phosphonium cation-based MPI agents have been developed (Fig. 1). The first type of ^{18}F–labeled phosphonium cations, such as [^{18}F]FBnTP [18,19], [^{18}F]FTPP [20], [^{18}F]FMBTP and [^{18}F]mFMBTP [21] were designed based upon the construction of tetraphenylphosphonium (TPP), in which a fluoro-substituted phenyl or benzyl group was attached to triphenylphosphine for ^{18}F labeling. The other type [5,22–27], such

Abbreviations: MPI, myocardial perfusion imaging; SPECT, single photon emission computed tomography; PET, positron emission tomography; CAD, coronary artery disease; MMP, mitochondrial membrane potential; CCCP, carbonyl cyanide m-chlorophenylhydrazone; PBS, phosphate-buffered saline; rt, room temperature; TFA, trifluoroacetic acid; p.i., post-injection; RCP, radiochemistry purity.

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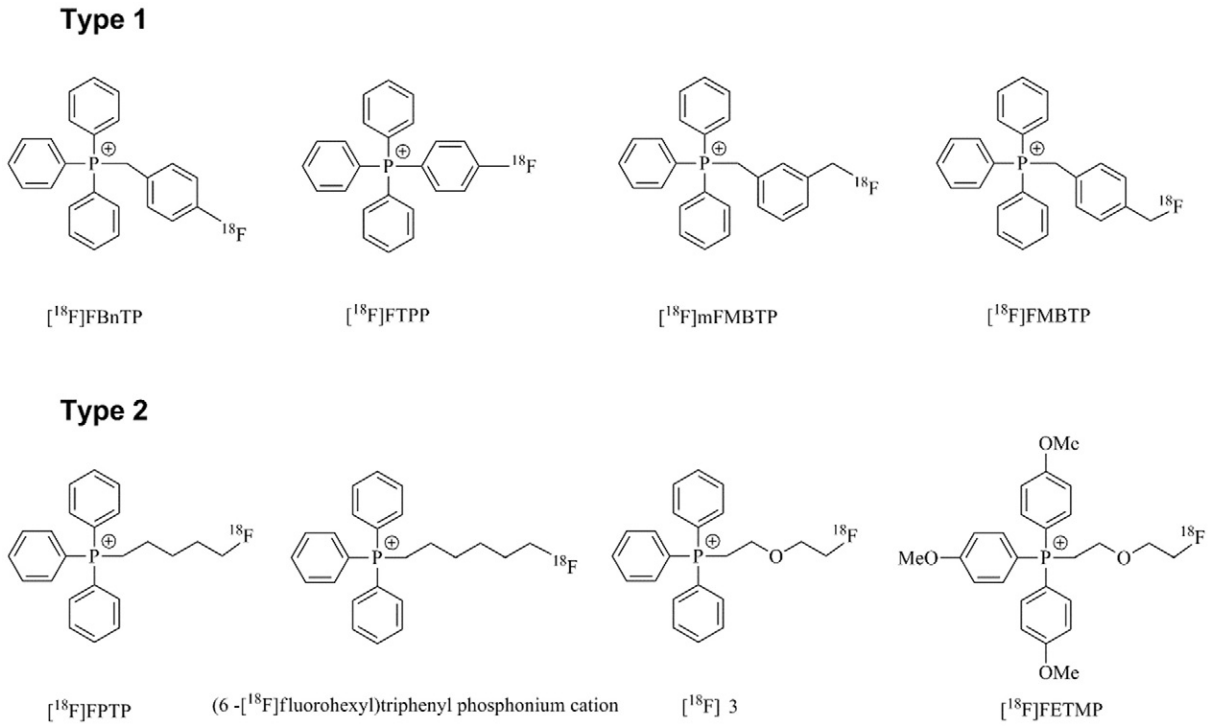


Fig. 1. Structure of reported ^{18}F–labeled lipophilic phosphonium cations as MPI agents. Type 1: a fluoro-substituted phenyl or benzyl group was attached to triphenylphosphine for ^{18}F labeling. Type 2: short alkyl or PET linkers were introduced between the triphenylphosphonium core and ^{18}F.

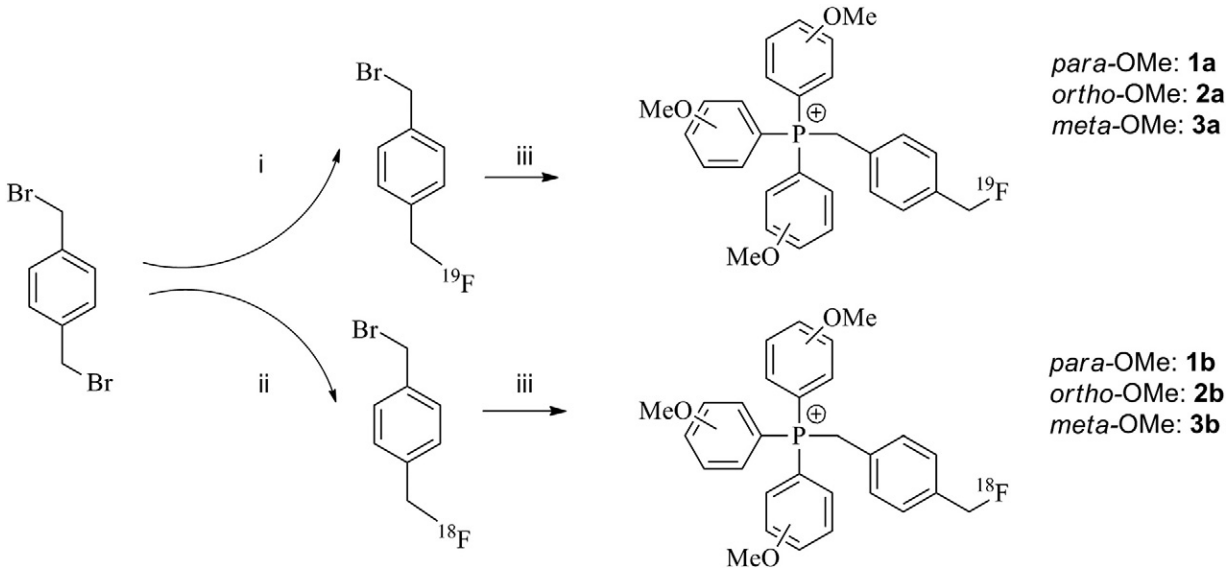
as [^{18}F]FPTP, [^{18}F]3 and [^{18}F]FETMP, were mainly reported by Kim et al., in which short alkyl or PEG linkers were introduced between the triphenylphosphonium core and ^{18}F to improve the heart uptake and excretion kinetics from non-targeting organs. In addition to the introduction of pharmacokinetic modifying (PKM) linkers, adding methoxy groups to the structure backbone might be of great interest in the design of MPI agents, since many widely used MPI agents contain methoxy groups, such as ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin.

In this study, three novel ^{18}F–labeled phosphonium cations, [^{18}F]4-(fluoromethyl)benzyltris(4-methoxyphenyl) phosphonium cation (**1b**), [^{18}F]4-(fluoromethyl)benzyltris(2-methoxyphenyl) phosphonium cation (**2b**) and [^{18}F]4-(fluoromethyl)benzyltris(3-methoxyphenyl) phosphonium cation (**3b**) were designed and synthesized based on the structure of [^{18}F]FMBTP that we previously reported [21] (Scheme 1). The methoxy groups were introduced into the phenyl groups of triphenylphosphine moieties on *para*, *ortho* and *meta* positions, respectively. The effect of methoxy-group position on myocardium uptake and pharmacokinetics properties of ^{18}F–labeled phosphonium cations was investigated.

2. Materials and methods

2.1. Reagents and instruments

Non-carrier-added [^{18}F]fluoride was supplied by PET center of Xuanwu Hospital (Beijing, China). Reagents and solvents were



Scheme 1. Synthetic route of **1a**, **2a**, **3a** and **1b**, **2b**, **3b**. (i) KF·2H$_2$O, CH$_3$CN, 18-crown-6; (ii) [^{18}F]KF/K$_{222}$, CH$_3$CN; (iii) tris(methoxyphenyl) phosphine, CH$_3$CN.