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Binding of 2-[^{18}F]fluoro-CP-118,954 to mouse acetylcholinesterase: microPET and ex vivo Cerenkov luminescence imaging studies☆

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Abstract

Acetylcholinesterase (AChE) has been an important cholinergic factor for the diagnosis of Alzheimer's disease (AD), because of reduced AChE activity in the postmortem brains of AD patients. We previously developed 5,7-dihydro-3-(2-(1-(2-[^{18}F]fluorobenzyl)-4-piperidinyl) ethyl)-6*H*-pyrrolo(3,2,f)-1,2-benzisoxazol-6-one (2-[^{18}F]fluoro-CP-118,954) for in vivo studies of AChE in mice. In the present study, we automated the synthesis of 2-[^{18}F]fluoro-CP-118,954 for the routine use and evaluated the radioligand by microPET and ex vivo Cerenkov luminescence imaging of mouse AChE. 4-[^{18}F]Fluoro-donepezil, another AChE inhibitor, was used for comparison. Automated syntheses of 2-[^{18}F]fluoro-CP-118,954 and 4-[^{18}F]fluoro-donepezil resulted in high radiochemical yields (25–33% and 30–40%) and high specific activity (27.1–35.4 and 29.7–37.3 GBq/μmol). Brain microPET images of two ICR mice injected with 2-[^{18}F]fluoro-CP-118,954 demonstrated high uptake in the striatum (ROI analysis: 5.1 %ID/g for the first 30 min and 4.1 %ID/g for another 30 min), and a blocking study with injection of CP-118,954 into one of the mice at 30 min after radioligand injection led to complete blocking of radioligand uptake in the striatum (ROI analysis: 1.9 %ID/g), whereas ^{18}F-labeled donepezil did not show specific uptake in the striatum. In another set of experiments, the brain tissues (striatum, parietal cortex, frontal cortex and cerebellum) were excised after brain microPET/CT imaging of mouse injected with 2-[^{18}F]fluoro-CP-118,954, and a high striatal uptake was also detected in ex vivo optical and microPET images (ROI analysis: 1.4 %ID/g) and in γ-counting data (2.1 %ID/g at 50 min post-injection) of the brain tissues. Taken together, these results demonstrated that 2-[^{18}F]fluoro-CP-118,954 specifically binds to AChE in mouse brains.

Keywords: Acetylcholinesterase; Alzheimer's disease; 2-[^{18}F]Fluoro-CP-118,954; 4-[^{18}F]Fluoro-donepezil; MicroPET imaging; Cerenkov luminescence imaging

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder [1] that is characterized by impairment of memory and cognition [2]. Early detection of AD would be beneficial for effective treatment, particularly when the drugs for the disease treatment but not symptomatic treatment are available. Several molecular targets are involved in the diagnosis of AD, including acetylcholinesterase (AChE), nicotinic acetylcholine receptor, muscarinic acetylcholine recep-tor, high affinity choline transporter, and β-amyloid plaques and neurofibrillary tangles. Of these targets, AChE, the enzyme that terminates cholinergic action through hydrolysis of acetylcholine to choline and acetate, has received considerable attention as an important cholinergic factor for the diagnosis of AD, because cortical choline acetyltransferase and AChE activity are significantly reduced in the postmortem brains of AD patients and AChE

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inhibitors are currently used for the symptomatic treatment of AD [3–6].

There are two strategies for in vivo studies of AChE: one is to use radiolabeled AChE substrates and the other is to use radiolabeled AChE inhibitors. The former strategy is used to measure AChE activity using radioligands, such as *N*-[^{11}C] methylpiperidin-4-yl acetate (MP4A or AMP) and *N*-[^{11}C] methylpiperidin-4-yl propionate (MP4P or PMP). Human PET imaging studies using theses radioligands showed high cortical AChE specificity (94% and 86%, respectively) and good correlations between AChE activity and k_3 values (R^2>0.98 and 0.99, respectively) [7–9]. However, this approach requires optimal pharmacokinetic analyses for the hydrolysis of radioligands by AChE in the brain. The latter strategy measures enzyme density using radiolabeled AChE inhibitors. Of the many radioligands developed for this purpose, *N*-benzylpiperidine derivatives have high anti-AChE activity and high selectivity for AChE, but their radiolabeled forms do not specifically bind to AChE in rodent brains [10–13]. In particular, the ^{11}C- and ^{18}F-labeled forms of 1-benzyl-4-((5,6-dimethoxy-1-oxoindan-2-yl)methyl)piperidine (donepezil; Aricept®), an FDA-approved drug for the treatment of AD, also showed uniform regional distribution in mouse brains [12,13]. However, *N*-benzylpiperidine lactam benzisoxazole derivatives, such as 5,7-dihydro-3-(2-(1-(phenylmethyl)-4-piperidinyl)ethyl)-6*H*-pyrrolo(3,2-f)-1,2-benzisoxazol-6-one (CP-118,954) and its methylated form (CP-126,998), showed excellent anti-AChE activity (IC_{50}=0.33 and 0.48 nM, respectively) and high selectivity for AChE [14]. ^{11}C-Labeled CP-126,998 showed high and specific binding to AChE in mouse brains, which had a maximum target (striatum) to non-target (cerebellum) uptake ratio of 4.4 at 60 min postinjection [15]. In addition, there was a good correlation between a radioligand distribution volume from PET images of normal human subjects injected with [^{11}C]CP-126,998 and AChE concentration from human postmortem brains [16]. We developed ^{18}F-labeled radioligands based on CP-118,954, because ^{18}F has advantages over ^{11}C for the labeling of this ligand due to its longer half-life (109.8 min) than that of ^{11}C (20 min) and low positron energy. The radioligands exhibited high and specific binding to AChE in tissue distribution of mouse brains [17,18].

Recently, several groups reported the potential applications of Cerenkov radiation resulting from charged particles such as positron- and beta-emitting radioisotopes to optical imaging [19–23]. They showed that Cerenkov luminescence imaging results are consistent with those obtained by microPET imaging in rodents [20–23].

In this study, we automated the synthesis of 2-[^{18}F]fluoro-CP-118,954 and evaluated the radioligand by microPET and ex vivo Cerenkov luminescence imaging of AChE in the mouse brain.

2. Materials and methods

Chemicals and solvents were obtained from Sigma-Aldrich (Milwaukee, WI, USA). [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F reaction using a GE Healthcare PETtrace cyclotron (Uppsala, Sweden). Automated synthesis of radioligands was carried out using TRACERlab FX F-N (GE Healthcare, Uppsala, Sweden). TLC was performed using a Bioscan radio-TLC scanner (Washington DC, USA). HPLC was carried out using the SpectraSYSTEM (Thermo Electron Corp., Waltham, MA, USA) equipped with an analytical column (YMC-Pack C18, 5 μm, 4.6×250 mm). The eluant was simultaneously monitored using NaI(Tl) radioactivity and UV (254 nm) detectors. Radioactivity was measured in a dose calibrator (Biodex Medical Systems, Shirley, NY, USA) and tissue radioactivity was measured using a 2480 WIZARD^2 Automatic Gamma Counter (PerkinElmer, Waltham, MA, USA). Measurement of residual solvents in the final product solution was carried out using an Agilent 7890A gas chromatograph (Wilmington, DE, USA). MicroPET and microPET/CT images of the mice injected with the radioligands were acquired using an Inveon microPET/CT scanner (Siemens Medical Solutions, Malvern, PA, USA) and optical images of the mouse brain tissues were obtained using an IVIS Spectrum (Caliper Life Sciences, Hopkinton, MA, USA) at the Center for Molecular and Cellular Imaging, Samsung Biomedical Research Institute (Seoul, Korea).

2.1. Automated synthesis

Automated synthesis of 2-[^{18}F]fluoro-CP-118,954 and 4-[^{18}F]fluoro-donepezil was carried out using our previous manual synthesis methods with some modifications [13,18]. The ^{18}F-labeling was initiated by adding a solution of 2-trimethylammoniumbenzaldehyde triflate (3.3 mg) in DMSO to K[^{18}F]F. The reaction mixture was stirred at 95°C for 7 min and cooled to 40°C, and then a solution of CP-144,885 (3.5 mg), $NaBH_3CN$ (4.8 mg), acetic acid (2 μl) and DMSO (0.2 ml) was added. The mixture was stirred at 130°C for 15 min. At the end of the reaction, the reaction mixture was diluted with 10 ml of water and passed through a tC18 Sep-Pak cartridge. The crude product trapped in the tC18 Sep-Pak cartridge was eluted using 1.5 ml of MeOH and then injected into a semipreparative HPLC column (C18, 7 μm, 16×250 mm Macherey-Nagel, Duren, Germany) using a 70:30 mixture of 0.1 M ammonium formate–acetonitrile as the eluant (flow rate of 8 ml/min). The desired fraction, which eluted at 24–26 min, was collected in a dilution flask containing water. The diluted solution was transferred into a tC18 Sep-Pak cartridge, which was washed with 10 ml of water. The purified product was eluted from the cartridge using 1 ml of EtOH followed by 9 ml of saline and then collected in a sterile vial via a sterile filter (0.22 μm).

4-[^{18}F]Fluoro-donepezil was synthesized in a similar manner to 2-[^{18}F]fluoro-CP-118,954. The crude product was purified by HPLC using a 60:40 mixture of 0.1 M ammonium formate–acetonitrile as the eluant (flow rate of