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In vivo evaluation of [^{18}F]FECIMBI-36, an agonist 5-HT$_{2A/2C}$ receptor PET radioligand in nonhuman primate

Jaya Prabhakaran[a,b], **Kiran Kumar Solingapuram Sai**[c], **Francesca Zanderigo**[a,b], **Harry Rubin-Falcone**[a], **Matthew J. Jorgensen**[d], **Jay R. Kaplan**[d], **Katharine I. Tooke**[a], **Akiva Mintz**[c], **J. John Mann**[a,b], and **J. S. Dileep Kumar**[*,b]

[a]Department of Psychiatry, Columbia University Medical Center, New York, USA

[b]Molecular Imaging and Neuropathology Division, New York State Psychiatric Institute, New York, USA

[c]Department of Radiology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

[d]Department of Pathology, Section on Comparative Medicine, Wake Forest School of Medicine Winston-Salem, North Carolina, USA

Abstract

We recently reported the radiosynthesis and *in vitro* evaluation of [^{18}F]-2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-(2-fluoroethoxy)benzyl)ethanamine, ([^{18}F]FECIMBI-36) or ([^{18}F]**1**), an agonist radioligand for 5HT$_{2A/2C}$ receptors in postmortem samples of human brain. Herein we describe the *in vivo* evaluation of [^{18}F]FECIMBI-36 in vervet /African green monkeys by PET imaging. PET images show that [^{18}F]FECIMBI-36 penetrates the blood-brain barrier and a low retention of radioactivity is observed in monkey brain. Although the time activity curves indicate a somehow heterogeneous distribution of the radioligand in the brain, the low level of [^{18}F]FECIMBI-36 in brain may limit the use of this tracer for quantification of 5-HT$_{2A/2C}$ receptors by PET.

Graphical abstract

Keywords

5-HT; 5-HT$_{2A/2C}$R; agonist; PET; radiotracer

[*]Corresponding author: Fax: 617-774-7521; kumardi@nyspi.columbia.edu.

Serotonin 2A receptors (5-HT_2Rs) are a class of excitatory 5-HT receptor, belonging to the superfamily of G protein coupled receptors (GPCRs).[1–4] These receptors comprise of 3 subtypes namely 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R. Among these, 5-HT_{2A}R is the most abundant 5-HT receptor in the human brain and it is involved in the pathophysiology of a variety of neuropsychiatric and neurodegenerative diseases and in the aging of brain.[5–9] Agonists acting at 5-HT_{2A}R have been evaluated in the treatment of neuropsychiatric disorders. 5-HT_2Rs exist in an active high affinity agonist binding state and in an inactive state, in which the receptor binds to agonists with low affinity.[2–4] Due to the large receptor density and heterogeneous distribution in brain, 5-HT_{2A}R is an important target for brain imaging with PET.[10–14] Measuring the agonist binding to the functional high affinity state by PET would enable determination of the involvement of 5HT_2R in the pathology of neuropsychiatric diseases. PET imaging using agonist radioligands can also lead to diagnosis and aid the development of new agonist medications via occupancy measurement studies. The antagonist PET ligands developed so far are not proven successful for the quantification of high affinity confirmation of 5-HT_{2A}Rs.[12–14] [^{11}F]CIMBI-5 and [^{11}F]CIMBI-36, analogues of dimethoxyphenylethan-1-amine, are the two 5-$HT_{2A/2C}$ agonist radiotracers tested *in vivo* in pigs and baboons.[15–20] So far, and only [^{11}F]CIMBI-36 is currently being investigated in human.[21, 22] Although [^{11}F]CIMBI-5 and [^{11}F]CIMBI-36 show proof of concept of *in vivo* quantification of 5-HT_{2A}R by PET, slow washout in cortical regions, low cortex to cerebellum binding ratios and relatively short half life of [^{11}F] may limit their widespread use in clinical imaging studies. In view of circumventing these shortcomings, we recently developed FECIMBI-36 (**1**), a high affinity 5-$HT_{2A/2C}$R agonist ligand that can be radiolabelled with [^{18}F].[23] Compound **1** exhibits high affinity to 5-HT_{2A}R (Ki = 1 nM), 5-HT_{2B}R (Ki = 2.8 nM) and 5-HT_{2C}R (Ki = 1.7 nM).[23] FECIMBI-36 did not show significant affinity for any other brain receptors and biogenetic amines.[23] Functional studies reveal that FECIMBI-36 has an E_{max} of 63.4% for 5-HT_{2A}R, 18.6% for 5-HT_{2B}R and 100% for 5-HT_{2C}R.[23] *In vitro* autoradiography experiments demonstrate that [^{18}F]FECIMBI-36 labels 5-HT_{2A}R and 5-HT_{2C}R receptors in postmortem human brain sections.[23] Herein we describe the results of our *in vivo* PET imaging results of [^{18}F]FECIMBI-36 in vervet monkey.

Synthesis of radiolabeling precursor, reference standard FECIMBI-36 and [^{18}F]FECIMBI-36 were achieved by our recently reported method.[23] In short, radiosynthesis of [^{18}F]FECIMBI-36 was achieved by a one-pot two-step procedure by reacting *tert*-butoxycarbonyl (Boc) protected precursor with [^{18}F]fluoroethyl tosylate ([^{18}F]FEOTs) in presence of Cs_2CO_3, followed by removal of Boc group in 25±5% radiochemical yield and > 95% chemical and radiochemical purities. PET imaging was performed in fasted adult male vervet/ African green monkey (*Chlorocebus aethiops sabaeus*) monkey under anesthetic conditions.[24]

PET images show that although [^{18}F]FECIMBI-36 penetrates the blood-brain barrier (BBB), as indicated by Figure 2, the amount of radioligand accumulated in brain is low. Regional time activity curves (TACs) are shown in Figure 3. TACs show a somehow heterogeneous distribution of [^{18}F[CEIMBI-36 in monkey brain despite low SUV (~0.3 g/mL) in brain. The highest accumulation of radioactivity was found in cortical regions, followed by cerebellum

and hippocampus. Among the considered regions of interest, thalamus and putamen show the lowest activity of the radiotracer with relatively rapid washout. The TAC based binding ratios of occipital cortex, orbital cortex, parietal cortex, frontal cortex, amygdala, anterior cingulate, hippocampus, cerebellum, thalamus, caudate, to putamen were 6.5, 6.2, 4.9, 4.5, 3.7, 3.8, 2.5, 2.6, 1.7 and 1.6 respectively at 115 minutes. PET images also show binding of the tracer to white matters in brain. The ratio of cerebral and cerebellar grey to white matters based on TAC analyses were ~1.6 at 115 min.

Values of [^{18}F]FECIMBI-36 distribution volume were calculated in the different regions using graphical analysis (Logan plot)[25] and an arterial input function, which was not corrected for the presence of radiotracer metabolites (values of distribution volume therefore are here referred to as VT*). Such values are several fold lower than those for typical brain PET ligands, potentially due to low retention or low delivery of radiotracer in brain. Binding potential (BP_{ND}) values of [^{18}F]FECIMBI-36 were calculated using the simplified reference tissue model (SRTM)[26] and graphical analysis (reference Logan plot)[27] with putamen as reference region (Figure 4). Both methods show comparable BP_{ND} estimates. Cortical regions show higher BP_{ND} values than other regions and the results are in agreement with analyses based on SUV. The PET imaging result in monkeys shown above using [^{18}F]FECIMBI-36 is consistent with the recently reported PET imaging results in pigs regarding low uptake in brain.[28] Low brain uptake of [^{18}F]FECIMBI-36 may be partly due to its relatively higher lipophilicity (ClogP = 4.0)[23] and low proportion of high affinity agonist binding site of 5-HT_{2A}R in monkey brain.

In summary, PET studies in anesthetized monkey show that [^{18}F]FECIMBI-36 penetrates the BBB and exhibits binding in 5-HT_{2A}R enriched regions. Although the proof of concept of *in vivo* PET imaging using [^{18}F]FECIMBI-36 is demonstrated in monkey, the radioligand showed low brain uptake and VT* values, and thus may not be ideal for further *in vivo* quantification in brain. Therefore, we pursue more suitable analogues of dimethoxyphenylethan-1-amine or related small molecules to develop successful PET ligands for further investigations.

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24. PET imaging of [^{18}F]FECIMBI-36 in monkey: PET scan was performed in vervet monkey (7.76 kg) using a GE 64-slice PET/CT Discovery VCT Scanner (General Electric Medical Systems, Milwaukee, WI, USA) under anesthesia (1.5–2.0% isoflurane). An intravenous line was used for radiotracer injection and to maintain an infusion with 0.9% NaCl throughout the experiment. An arterial line was placed for obtaining arterial samples for calculation of the input function. After a 10 min transmission scan, [^{18}F]FECIMBI-36 was injected (2.65 mCi) as intravenous bolus over 30 seconds, and emission data were collected for 120 min in 3-dimensional mode. Arterial plasma samples were taken manually at 2, 4, 6, 8, 12, 20, 40, 80, 100 and 120 minutes after radiotracer injection. Plasma samples were analyzed using our established procedures to measure the total radiotracer radioactivity in arterial plasma over time.[29] Image analysis was performed in MATLAB 2012b (The Mathworks, Natick, MA, USA). Registrations from PET to magnetic resonance image (MRI) and MRI to standard space were performed with FMRIB Software Library (FSL) linear registration module FLIRT.[30] Time activity curves (TACs) were then extracted as the mean PET values within each region of interest based on the INIA Primate atlas.[31]
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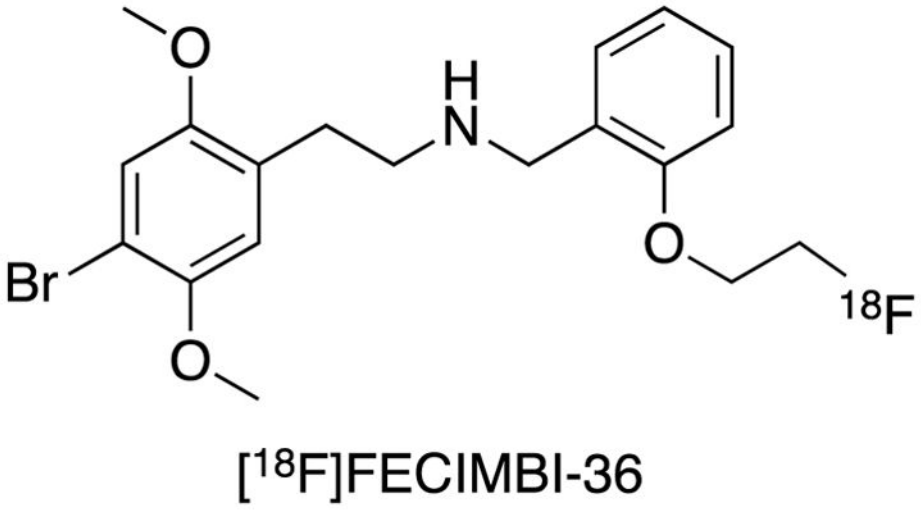

Figure 1.
Chemical structure of [^{18}F]FECIMBI-36

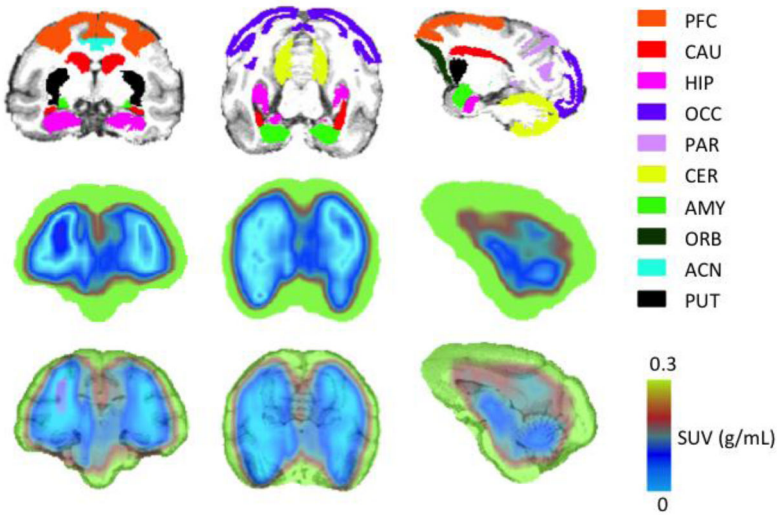

Figure 2.

Ten key brain regions labels in magnetic resonance imaging (MRI) template (Row 1), PET standard uptake value (SUV) (using the sum of radioactivity over a 120 minute scan) (Row 2), and MRI-PET SUV co-registered images (Row 3) of [^{18}F]FECIMBI-36 in vervet monkey brain; Column 1: Axial; Column 2: Coronal; Column 3: Sagittal. (ACN: anterior cingulate; AMY: amygdala; CAU: caudate; CER: cerebellar grey matter; PFC: prefrontal cortex; HIP: hippocampus; OCC: occipital cortex; ORB: orbital cortex; PAR: parietal cortex; PUT: putamen)

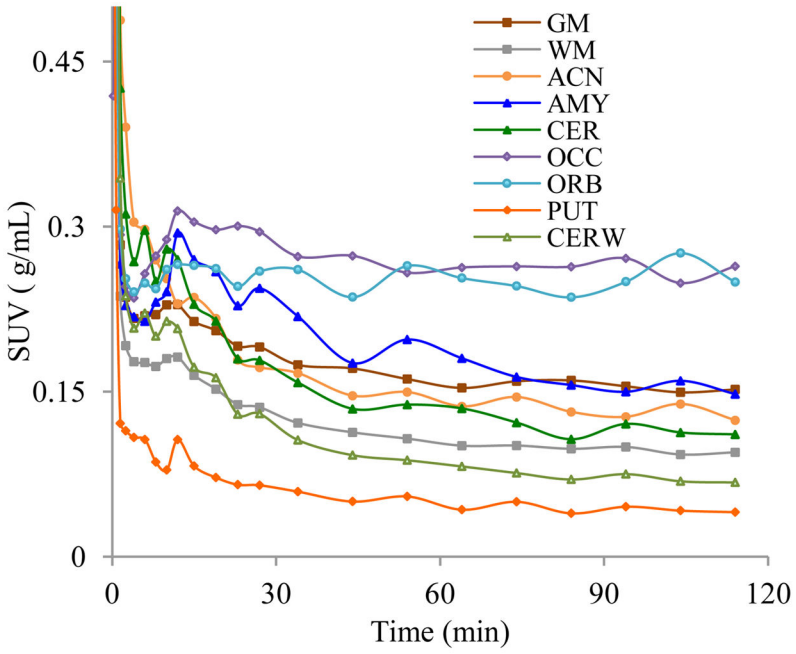

Figure 3.

TACs of [^{18}F]FECIMBI-36 in vervet monkey brain (ACN: anterior cingulate; AMY: amygdala; CER: cerebellar grey matter; CERW: cerebellar white matter; OCC: occipital cortex; ORB: orbital cortex; GM: total cerebral grey matter; PUT: putamen; WM: Cerebral white matter).

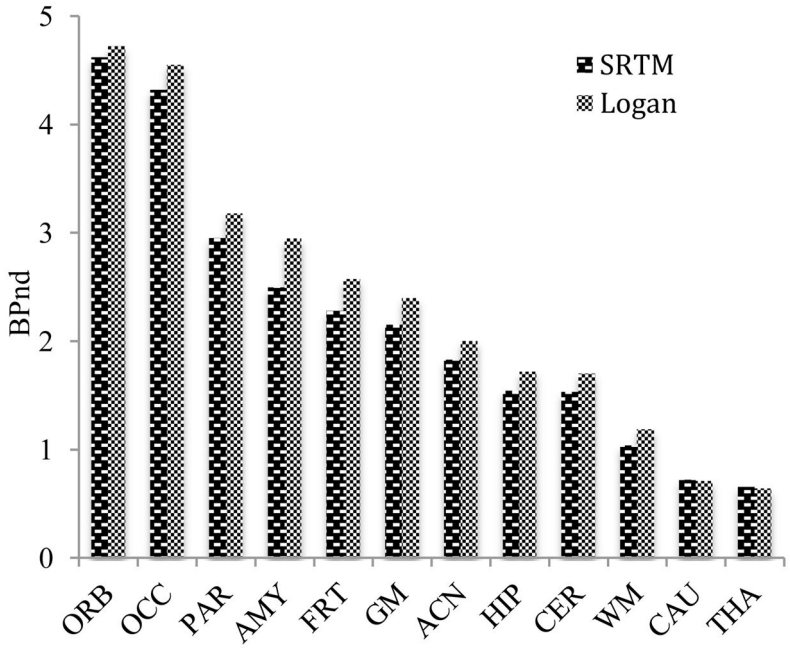

Figure 4.
Binding potential (BP_{ND}) of [^{18}F]FECIMBI-36 in vervet monkey brain regions.