First-In-Human *in Vivo* Imaging and Quantification of Monoacylglycerol Lipase in The Brain: A PET Study With ^{18}F -T-401

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Abstract

Purpose: Monoacylglycerol lipase (MAGL) regulates cannabinoid neurotransmission and the pro-inflammatory arachidonic acid pathway by degrading endocannabinoids. MAGL inhibitors may accordingly act as cannabinoid-potentiating and anti-inflammatory agents. Although MAGL dysfunction has been implicated in neuropsychiatric disorders, it has never been visualized *in vivo* in human brain. The primary objective of the current study was to visualize MAGL in the human brain using the novel PET ligand ^{18}F -T-401.

Methods: Seven healthy males underwent 120-min dynamic ^{18}F -T-401-PET scans with arterial blood sampling. Six subjects also underwent a second PET scan with ^{18}F -T-401 within 2 weeks of the first scan. For quantification of MAGL in the human brain, kinetic analyses using one- and two-tissue compartment models (1TCM and 2TCM, respectively), along with multilinear analysis (MA1) and Logan graphical analysis were performed. Time-stability and test-retest reproducibility of ^{18}F -T-401-PET were also evaluated.

Results: ^{18}F -T-401 showed rapid uptake and gradual washout from the brain. Logan graphical analysis showed linearity in all subjects, indicating reversible radioligand kinetics. Using a metabolite-corrected arterial input function, MA1 estimated regional total distribution volume (V_T) values by best identifiability. V_T values were highest in the cerebral cortex, moderate in the thalamus and putamen, and lowest in white matter and the brainstem, which was in agreement with regional MAGL expression in the human brain. Time-stability analysis showed that MA1 estimated V_T values with a minimal bias even using truncated 60-min scan data. Test-retest reliability was also excellent with the use of MA1.

Conclusions: Here, we provide the first demonstration of *in vivo* visualization of MAGL in the human brain. ^{18}F -T-401 showed excellent test-retest reliability, reversible kinetics, and stable estimation of V_T values consistent with known regional MAGL expressions. PET with ^{18}F -T-401-PET is promising tool for measurement of central MAGL.

Introduction

The endocannabinoid system is responsible for intercellular signaling involved in a wide variety of physiological processes, including regulation of cardiovascular, gastrointestinal, and brain functions. In the central nervous system (CNS), endocannabinoid signaling is associated with synaptic plasticity modulations of other transmitters in neurons and cytokine release from microglia, and hence is implicated in diverse neurological and psychiatric disorders. Endocannabinoid transmission is mediated by the two endocannabinoids, 2-arachidonolglycerol (2-AG) and anandamide, and endocannabinoid receptors 1 and 2 (CB1 and CB2, respectively). In the CNS endocannabinoid system, 2-AG is considered to play more essential roles than anandamide [1]. Although the binding affinity of 2-AG for the endocannabinoid receptors was lower ($K_i = 99 \text{ nM}$) than that of anandamide ($K_i = 2.4 \text{ }\mu\text{M}$) [2], concentrations of 2-AG were observed to be approximately 800 times higher than those of anandamide in the rat brain [3]. Furthermore, 2-AG, but not anandamide, is primarily released from the postsynaptic component to mediate retrograde signaling through the presynaptic CB1 [4, 5]. Thus, 2-AG is conceived to be a pivotal molecule in endocannabinoid neurotransmission.

Monoacylglycerol lipase (MAGL) is a 33-kDa cytosolic serine hydrolase enzyme that cleaves monoacylglycerol into fatty acids and glycerol. MAGL is the main catalyst of 2-AG hydrolysis in the brain, accounting for 85% of 2-AG hydrolase activities [6]. It has been reported that inhibition of MAGL increases the brain levels of 2-AG [6, 7], leading to the activation of endocannabinoid neurotransmission and consequent antinociceptive, anxiolytic and antiemetic responses through CB1 receptor [8]. In addition, the hydrolysis of 2-AG by MAGL leads to the production of arachidonic acid (AA) and its downstream metabolite prostaglandins, which accelerate neuroinflammation in the brain [6]. MAGL inhibition has been shown to decrease arachidonic acid levels [9], resulting in anti-inflammatory effects in the brain [10] as well as neuroprotective effects [11]. Thus, MAGL could be a promising target for drug development for neuropsychiatric disorders in which the endocannabinoid system and neuroinflammation are implicated.

While considerable efforts have been made to develop a specific radioligand for imaging of MAGL by positron emission tomography (PET), *in vivo* visualization of MAGL in the human brain had yet to be enabled. Several PET imaging compounds for MAGL have been applied to laboratory animals [12–14], but the slow kinetics of these radioligands in the brain imply their irreversible binding to MAGL, impeding the quantification of the binding components. Hence, a reversible PET tracer is required for robust evaluations of MAGL density and its occupancy by a MAGL inhibitor.

We recently developed a radiofluorinated derivative of 5((4R)-1-{3-[2- ^{18}F -fluoro-4-methylpyridin-3-yl]phenyl}-4-[4-(1,3-thiazol-2-ylcarbonyl)piperazin-1-yl]pyrrolidin-2-one, ^{18}F -T-401, as a PET ligand for assessing MAGL in the living brain. In our previous study, ^{18}F -T-401 autoradiography of the rat and rhesus monkey brains showed abundant specific binding of the radioligand in the neocortex and hippocampus in contrast to its weak binding in the brainstem, which was in agreement with the anatomical distribution of MAGL in the brains of these animals [15]. In addition, PET imaging of rats and rhesus monkeys demonstrated that ^{18}F -T-401 specifically binds to MAGL with adequate reversibility [16]. Furthermore, pre-administration of unlabeled T-401 successfully blocked the uptake of radioactivity in a dose-dependent manner, supporting the binding saturability of ^{18}F -T-401 in rat and monkey brains. In addition, pretreatment of a monkey with a MAGL inhibitor, JW642, gave rise to declines of ^{18}F -T-401 retention in a dose-dependent manner, providing evidence for the specificity of this radioligand for MAGL [16]. These preclinical data suggested promising characteristics of ^{18}F -T-401 for *in vivo* PET imaging of MAGL in the human brain.

In the present study, we conducted the first-in-human study using ^{18}F -T-401 to visualize MAGL in the human brain. We also evaluated the safety and radioligand kinetics in the brain as well as the reproducibility of ^{18}F -T-401.

Materials And Methods

1. Radiopharmaceutical preparation

The chemical structure of ^{18}F -T-401 was described in a previous paper (75). ^{18}F -T-401 was prepared by ^{18}F -fluorination of a nitro precursor with [^{18}F]KF/Kryptofix 222 (Merck) according to previously reported procedures [15].

The injected radioactivity of ^{18}F -T-401 ranged from 183.5 to 197.2 MBq (189.8 ± 4.4 MBq, mean $\pm$ SD), and the molar activity of the radioligand at the time of injection ranged from 125.5 to 391.3 GBq/ μmol (207.7 ± 70.5 GBq/ μmol). The injected mass accordingly ranged from 0.5 to 1.6 nmol (1.0 ± 0.3 nmol).

2. Subjects

The study protocol was approved by the Radiation Drug Safety Committee and the Institutional Review Board of the National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan. Written informed consent was obtained from each subject after a complete explanation of the study. The current study was registered with the Japan Registry of Clinical Trials (jRCTs031180045).

Eight healthy male subjects participated in this study. Since one subject was excluded due to an uncorrectable body motion during the PET scan, data obtained from seven subjects with their ages ranging from 22 to 35 years (26.4 ± 3.7 years) were analyzed. All subjects enrolled in the present study were confirmed to have no history of current or past physical and psychiatric illnesses, substance abuse, or any organic brain diseases based on their medical history, a physical examination, blood and urine analyses, and magnetic resonance (MR) imaging of the brain. To evaluate the safety of ^{18}F -T-401, blood tests, including a complete blood cell count and serum biochemistry, were conducted before and 2 h after injection of ^{18}F -T-401.

3. Measurement of ^{18}F -T-401 in plasma

To obtain arterial input functions, arterial blood samples were taken manually 35 times after the injection of ^{18}F -T-401 as described in Supplementary Methods.

The fractions of the parent radioligand and its radiometabolites in plasma were determined by high-performance liquid chromatography (HPLC) as described in Supplementary Materials.

4. PET and MR scan procedures

All PET scans were performed with a Biograph mCT Flow system (Siemens Healthcare, Erlangen, Germany) as previously described [17]. To evaluate the test-retest variability, five subjects underwent the second PET scan within 2 weeks of the first PET scan. The first PET scan was conducted under the condition of no food intake. The second PET scan was performed with the condition of no food intake for three subjects, but with food intake for the other three subjects.

MR imaging was performed under the same conditions as in our previous study [17]. None of the subjects exhibited apparent structural abnormalities in their MR images.

5. Brain image processing

Reconstructed dynamic data were realigned for motion correction according to the process of frame-to-reference image registration in PMOD (version 4.0; PMOD Technologies Ltd.). These motion-corrected dynamic PET images were coregistered to T1-weighted MR images.

All T1-weighted MR images were automatically segmented using FreeSurfer 6.0, which is documented and freely available for download online (<http://surfer.nmr.mgh.harvard.edu/>). Regional time-activity curves were extracted from the following volumes of interest [18–20]: the cerebellar cortex, thalamus, caudate, putamen, hippocampus, frontal lobe, temporal lobe, parietal lobe, occipital, cingulate, cerebral white matter, midbrain, and pons.

6. Estimation of pharmacokinetic parameters

The regional total distribution volume (V_T) [21], a combined index of the radioligand uptake and binding that equals the ratio between the concentrations of radioligand in tissue and plasma at equilibrium, was calculated. The radioligand in tissue exists in specifically bound and non-displaceable (nonspecifically bound and free) states. V_T values were estimated by analyzing the tissue and metabolite-corrected plasma time-radioactivity curves by compartment and graphical models.

We utilized graphical analyses, including Logan's graphical plot [22] and multilinear analysis (MA1) [23], with the cerebral blood volume contribution fixed at 5% for the estimation of V_T . First, the whole brain time-radioactivity data were used to determine t^* (min), when the system reaches transient equilibrium between the brain and plasma compartments as well as within the plasma compartment. Next, the V_T values in all brain regions were calculated using this uniform t^* . V_T was also determined with a one-tissue compartment model (1TCM) by assuming a single compartment for describing the radioligand kinetics in the brain and a two-tissue compartment model (2TCM) incorporating two compartments for specific binding and non-displaceable uptake of the radioligand in the brain. The kinetic assays were carried out with a radiometabolite-corrected plasma input function and a cerebral blood volume contribution fixed at 5%. The delay between the arrival of ^{18}F -T-401 in the radial artery and brain was also estimated with a 1TCM analysis of the tissue time-radioactivity data obtained from the whole brain but excluding the white matter at 0–10 min after the injection, along with the plasma measures. An optimal compartment model was chosen on the basis of Akaike information criterion (AIC) [25], Schwarz criterion (SC) [24], model selection criterion (MSC) [26], and the goodness of fit assessed by F statistics [27].

7. Time-stability analysis

To determine the minimal scan length for reliable quantification of V_T , and to assess whether ^{18}F -T-401 radiometabolites accumulate in the brain, the time stability of V_T estimation by an appropriate analytical model selected by the above-mentioned comparisons was examined by progressively truncating the 120-min scan by 10-min increments to the shortest length of 30 min.

8. Assessment of test-retest reproducibility

Test–retest reproducibility was assessed by calculating intrasubject variability and test–retest reliability (Supplementary Methods).

9. Assessment of the relationship between of ^{18}F -T-401 V_T values and regional mRNA expression levels of MGLL

To examine whether ^{18}F -T-401 PET captures MAGL in the human brain, we examined the relationship between V_T values of ^{18}F -T-401 and mRNA expression levels of MGLL in the human brain using data obtained in the Human Protein Atlas. Details are provided in Supplementary Methods.

10. Statistical analysis

Statistical analyses were conducted in SPSS (IBM SPSS Statistics version 26.0). For correlational analysis, Pearson correlation coefficients were calculated with two-tailed tests of significance.

Results

1. Safety

There were no adverse effects from the ^{18}F -T-401 injection. The subjects did not complain of any subjective symptoms during or after the PET scans with ^{18}F -T-401. In addition, the injection of ^{18}F -T-401 did not cause any changes in blood tests or vital signs.

2. Radioligand uptake in the brain

Time-activity curves in the brain following the ^{18}F -T-401 administration indicated rapid uptake and subsequent gradual washout of the radioactivity (Figure 2a). Peak brain uptake was observed within a few minutes after the ^{18}F -T-401 injection. The highest radioactivity uptake was observed in the cerebral cortex with SUV of 2.0. Although washout of radioactivity was noted in all brain regions, variability of radioactivity retention across these areas remained at 120 min after the ^{18}F -T-401 injection (Fig. 2). Washout was fastest in the brainstem, and was slowest in the cerebral cortex and cerebellum, where the density of MAGL expression is the highest.

3. Plasma radioactivity analysis

In plasma and whole blood, radioactivity showed a rapid increase, peaking at 20 to 40 standardized uptake value (SUV, radioactivity concentration divided by injected radioligand dose divided by body weight), and then a sharp decline followed by a rather persistent retention after administration (Figure 1a). About 40% of the plasma radioactivity was derived from unmetabolized ^{18}F -T-401 at 60 min (Figure 1b). Radiometabolites of ^{18}F -T-401 in plasma appeared slowly, then becoming the dominant source of plasma radioactivity at 90 min. Five radiometabolites ($M_1 - M_5$) of ^{18}F -T-401 appeared in plasma immediately after the radioligand injection and were present at all time points. The most abundant radiometabolite (M_1) was presumed to be more hydrophilic than the parent radioligand in light of its retention time in HPLC charts (Figure 1c). Two radiometabolites (M_4 and M_5) were observed to be more lipophilic than the parent radioligand, as they displayed longer retention time in HPLC, but their plasma levels remained low until 120 min. The plasma free fraction of ^{18}F -T-401 was $7.9 \pm 0.7\%$.

4. Radioligand kinetics in the brain

Kinetic analysis using metabolite-corrected arterial plasma input functions revealed that the estimation of V_T was robust and that the V_T values were estimated with variations in each brain region. Using 120-min data collected from 7 subjects, 2TCM was found to be better than 1TCM for describing the radioligand kinetics in the brain. Compared to 1TCM, 2TCM showed lower mean AIC scores (82.0 vs. 31.6) and higher mean MSC scores (2.5 vs. 3.8). An F test also showed that the goodness of the 2TCM fit was significantly higher than that of the 1TCM fit in almost all 182 fittings over a total of 13 scans ($p < 0.001$). All kinetic parameters determined with 2TCM are shown in Table 1. With 2TCM, V_T values were determined with a standard error of about 2.65%.

In the quantification of V_T by Logan graphical analysis, fair linearity after $t^* = 20$ min was shown in all subjects (Figure 3a). The linearity in Logan graphical analysis indicates that ^{18}F -T-401 has reversible binding properties, and that the accumulation of radiometabolites of ^{18}F -T-401 in the brain is negligible. The distinct non-zero k^* values at 20 min agreed with the findings that the kinetics of ^{18}F -T-401 were not well described by 1TCM, in which k^* is zero. V_T values estimated by Logan and MA1 graphical plots showed significant correlations with those estimated by 2TCM ($r = 0.99$, $p < 0.0001$ for both correlations) (Figure 4). Mean error in the estimation of V_T values for all brain regions was 0.76% for the Logan plot, and 0.05% for MA1. These results indicate that MA1 presents the best identifiability in estimating V_T values.

Although V_T values estimated by MA1 showed considerable inter-subject variability (17.9% - 22.2%), the rank order of regional V_T values of ^{18}F -T-401 showed excellent consistency across subjects. Regional V_T values determined by MA1 were between 1.1 and 3.3 in most fits and were highest in the cerebral cortex and cerebellum, moderate in the thalamus and putamen, and lowest in white matter and the brainstem (Figure 5). The V_T values calculated by MA1 were closely correlated with SUVs 60-120 min after injection ($p < 0.001$, $r > 0.99$ in all brain regions). There was no significant correlation between the mass of

radioligand administered and V_T values in all regions ($p > 0.05$), suggesting the unlikelihood that differences between subjects are caused by radioligand dosage.

5. Time-stability analysis

Time-stability analysis showed that V_T values estimated by MA1 were stable in the stepwise truncation of the dynamic data from 120 to 60 min and were well identified ($SE < 5\%$) (Figure 6). MA1 V_T values showed an average negative bias of $-2.0\% \pm 2.2\%$ and $-3.8\% \pm 3.7\%$ when the 120-min dynamic data were truncated to 90- and 60-min data, respectively. 2TCM and Logan graphical analysis also showed stable estimation of V_T values in the truncation of the dynamic data from 120 to 60 min (Supplementary Figure 3A, B).

6. Test-retest reproducibility

V_T values estimated by MA1 in the first and second PET sessions showed excellent test-retest reproducibility in all brain regions (Supplementary Figure 2). Likewise, 2TCM and the Logan graphical model yielded V_T values with high reproducibility between the test and retest scans. The average regional test-retest variability and ICC of estimated V_T values were 7.97% and 0.98 for MA1, 9.32% and 0.95 for 2TCM, 7.8%, and 0.98 for Logan graphical analysis, respectively. To examine the effect of food intake, we compared V_T values between conditions with and without dietary intake in the same subjects ($N=3$). There was no significant difference between V_T values between conditions with and without dietary intake ($p>0.05$).

7. Assessment of the relationship between of ^{18}F -T-401 V_T values and regional expression levels of MGLL mRNA

The V_T values estimated by MA1 and normalized expression level of MGLL were well correlated (nonparametric $p = 0.0067$ and $r = 0.9286$ for both). (Figure 7)

Discussion

In the current study, we report the first *in vivo* imaging of MAGL in the human brain using ^{18}F -T-401 PET. Brain uptake of ^{18}F -T-401 was sufficiently high with reversible kinetics and heterogeneous distribution. Using a metabolite-corrected arterial input function, MA1 accurately estimated V_T in multiple brain regions. The rank order of regional V_T values of ^{18}F -T-401 showed excellent inter-subject reliability and consistency with previously reported MAGL density in the human brain. ^{18}F -T-401 PET data demonstrated excellent reproducibility, and the scan length could be shortened to 60-90 min. These results supported the utility of ^{18}F -T-401 PET as a promising tool for the development of novel drugs targeting MAGL as well as for MAGL quantification in neurodegenerative and psychiatric diseases.

Utility of ^{18}F -T-401 PET as *in vivo* imaging of MAGL in the human brain

MAGL has been a target of drug development because it plays a crucial role in regulating endocannabinoid and prostaglandin signaling that modulates neuroinflammatory and neurodegenerative processes in various neuropsychiatric diseases. Although several PET drug candidates for visualizing MAGL have been reported in animal studies, all have only been used in preclinical studies [12,14,25]. Here we developed a PET ligand to visualize MAGL *in vivo* in the human brain. In the present study, V_T was estimated with excellent identification between subjects. We found that V_T values of ^{18}F -T-401 are highest in the cerebral cortex and lowest in the brainstem, consistent with previous autoradiographies of the mouse brain with ^{18}F -T-401 [15] and ^{11}C -SAR127303[25], and the rhesus monkey brain with ^{18}F -T-401 [16]. Furthermore, regional V_T values estimated by MA1 showed excellent correlation with the mRNA expression level of MGLL obtained from the Human Protein Atlas (Figure 7). These results indicate that PET with ^{18}F -T-401 can accurately quantify the density of MAGL in the human brain.

Kinetics of ^{18}F -T-401 in the human brain

Although several PET ligands for MAGL imaging have been developed [13,14,25], there was no PET ligand with reversible binding to MAGL. In general, irreversible PET ligands are accompanied by technical challenges that include difficulties in determining binding parameters, longer scan durations due to slow dissociation, and increased susceptibility to flow/delivery limitation effects caused by an excessively high affinity or trapping rate. In this study, the following data show that ^{18}F -T-401 has reversible binding properties to MAGL. First, the time-activity curve of ^{18}F -T-401 clearly showed rapid uptake into the brain and a gradual washout. Second, the linearity of Logan graphical analysis and stability of the time-stability analysis of 2TCM supports the reversible kinetics of ^{18}F -T-401 in the human brain. Consistently, binding parameters of ^{18}F -T-401 were estimated successfully with reversible models. Thus, we present the first, to our knowledge, *in vivo* evaluation and characterization of reversible PET ligand for MAGL imaging in healthy human volunteers.

Optimal kinetic model of ^{18}F -T-401 to quantify MAGL in the human brain

In selecting the best analytical method, we compared 1TCM, 2TCM, Logan graphical analysis and MA1. We identified MA1 as the preferred model for quantifying MAGL based on the standard error in estimating V_T and the results of time stability analysis. Correlation analysis showed that V_T values estimated by MA1, Logan graphical analysis and 2TCM were highly correlated with each other, and these models were equally practical. Based on time-stability analysis, we conclude that 60-min data are sufficient for quantifying MAGL. Since these results were obtained in healthy controls under drug-free condition, optimal kinetic model and scan length would need to be re-examined in diseased conditions or after drug administrations.

Effects of radiometabolites of ^{18}F -T-401 in the human brain

Our data showed that 5 radiometabolites of ^{18}F -T-401 appeared in plasma after injection. The most abundant radiometabolite was more hydrophilic than the parent radioligand in terms of their retention times in HPLC charts. Two radiometabolites (M4 and M5) were determined to be more lipophilic than the parent radioligand. However, their compositions in plasma remained sufficiently low until 120 min. Although we did not directly assess the binding of radioactive metabolites to MAGL, our previous study in monkeys indicated that the administration of an MAGL inhibitor produced irreversible compartments with radioactive metabolites in the brain, and therefore 3TCM was presumed to be a better kinetic model [16]. Considering the possibility that ^{18}F -T-401 is metabolized and accumulated in the human brain, we performed an additional analysis. We modified 2TCM by incorporating a compartment reflecting a radiometabolite converted irreversibly from ^{18}F -T-401 in the brain (Supplementary Methods and Supplementary Figure 1). Estimation of Compared to 2TCM, 3TCM showed slightly unstable estimation with a standard error of 5.5% (Supplementary Table 1), probably due to the instability of micro-parameter estimations such as k_5 . Time-stability analysis showed that V_T values estimated by 3TCM gradually and progressively increased with the length of the scans (Supplementary Figure 3C). These results indicate that 3TCM is not suitable for describing the ^{18}F -T-401 kinetics in a condition without administration of an MAGL inhibitor.

Assessments of the reproducibility of PET measurements with test-retest scans

In the present study, test-retest reproducibility of ^{18}F -T-401 was examined in 6 subjects. V_T values with MA1 of scan 1 and scan 2 showed excellent test-retest reproducibility (Supplementary Figure 2A). Reproducibility of MA1 V_T was good in the cerebral cortex (3.5% for frontal cortex) and the subcortical structures (3.5% for putamen and 3.5% for thalamus). There was somewhat greater variability of V_T in the brain stem (14.4% for pons and 7.6% for midbrain in 2TCM) where MAGL density is estimated to be low. Similarly, a high level of test-retest reliability was obtained with 2TCM and Logan graphical analysis (Supplementary Figure 2B, C). Although the estimated V_T values showed considerable inter-subject variability (17.9% - 22.2%), a consistent trend of regional differences in V_T values across subjects indicates that such variability reflects individual differences of MAGL density. High reproducibility of PET measures is critically important for the occupancy study of MAGL-targeting drugs and evaluation of longitudinal alteration of MAGL density in pathological conditions.

Effects of food intake on ^{18}F -T-401 binding measures

It has been repeatedly shown that endocannabinoid signaling is causally associated with food intake behavior and energy balance [26]. An observational study has shown the association of dietary patterns with endocannabinoid levels in overweight and obese subjects. Furthermore, experiments using CB1 receptor-deficient mice have shown that dysfunction of MAGL leads to abnormalities in food intake [27]. These findings suggest the possibility that ^{18}F -T-401 binding measures can be altered by food intake. To examine this notion, we compared V_T values between conditions with and without dietary intake in the same subjects (N=3). Our results indicate that the MAGL level in the brain is not altered by food intake ($p>0.05$).

Conclusions

The novel radioligand ^{18}F -T-401 showed optimal properties for *in vivo* imaging of MAGL in the human brain. To our knowledge, ^{18}F -T-401 is the first PET ligand for MAGL with reversible kinetics. MA1, 2TCM and Logan graphical analysis revealed the kinetics of ^{18}F -T-401 in the human brain with reliability, and with consistency with known MAGL distribution in the human brain. ^{18}F -T-401 is a suitable PET ligand for the quantification of MAGL in the human brain.

Declarations

Competing Interests and Funding

MH has received grant support from Takeda Pharmaceutical Company Limited. All other authors declare that they have no conflicts of interest. The precursor and reference standard of ^{18}F -T-401 for the present study were provided by Takeda Pharmaceutical Company Limited. This study was supported in part by a Grant-in-Aid for Scientific Research C (20K07935), a grant from the General Insurance Association of Japan (2020-2021) to KT, a Grants-in-Aid for Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS; 21dm0207072h0003), Strategic International Brain Science Research Promotion Program (Brain/MINDS Beyond; 20dm0307105h0002), JST Grant Number JPMJMS2024 and AMED Grant Number 20356533 to MH.

Author contribution

Keisuke Takahata and Makoto Higuchi contributed to conception and design, acquiring, analyzing and interpreting data, and drafting, revising the manuscript. Keisuke Takahata, Chie Seki, Manabu Kubota, Yasunori Sano, Yasuharu Yamamoto, Kenji Tagai, Hitoshi Shimada, Soichiro Kitamura, Kiwamu Matsuoka, Hironobu Endo, Hitoshi Shinotoh participated in data acquisition. Kazunori Kawamura and Ming-Rong Zhang were in charge of radioligand synthesis. Yasuyuki Kimura and Masanori Ichise contributed to analyzing and interpreting data. The first draft of the manuscript was written by Keisuke Takahata and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare no competing interests.

Ethics approval and consent to participate

This study was approved by the Radiation Drug Safety Committee, and the Institutional Review Board of National Institutes for Quantum Science and Technology, Chiba, Japan, and was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. After complete description of the study, written informed consent was obtained from all participants.

Consent for publication

Written informed consent was obtained from all participants regarding publishing their data.

Conflict of interest

The authors declare no competing interests

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Tables

Table 1. Parameters estimated for ¹⁸F-T401 PET data with 2TCM in the human brain

Kinetic rate constants (K_1 , k_2 , k_3 and k_4) and total distribution volume (V_T) from 7 subjects are shown as mean ± SD. Brain regions are listed, as are median standard errors (SE) expressed as % of the variable itself. K_1 [ml/ccm/min] and k_2 [1/min] are the uptake and clearance rate constants, whereas k_3 [1/min] and k_4 [1/min] describe the exchange between the tissue compartments. $V_T (=K_1/k_2*(1+k_3/k_4))$ represents the total distribution volume of tracer in tissue.

Regions	K_1 [ml/ccm/min]	SE [%]	k_2 [1/min]	SE [%]	k_3 [1/min]	SE [%]	k_4 [1/min]	SE [%]	k_3/k_4	SE [%]	V_T [ml/ccm]	SE [%]
Cerebellum	0.14 ± 0.04	3.59	0.12 ± 0.03	10.27	0.06 ± 0.01	14.13	0.03 ± 0.00	6.55	1.93 ± 0.30	10.36	3.39 ± 0.97	1.40
Thalamus	0.11 ± 0.04	3.40	0.10 ± 0.03	11.55	0.06 ± 0.02	19.45	0.04 ± 0.00	7.94	1.46 ± 0.48	14.36	2.72 ± 0.69	1.11
Caudate	0.12 ± 0.04	3.90	0.11 ± 0.08	18.48	0.09 ± 0.06	40.07	0.07 ± 0.02	13.33	1.24 ± 0.94	30.78	2.65 ± 0.74	0.90
Putamen	0.13 ± 0.04	2.89	0.09 ± 0.03	13.26	0.06 ± 0.03	33.50	0.07 ± 0.02	11.96	0.83 ± 0.34	24.29	2.87 ± 0.82	0.77
Hippocampus	0.14 ± 0.08	6.12	0.36 ± 0.43	17.12	0.18 ± 0.18	17.32	0.04 ± 0.01	6.94	4.62 ± 4.36	15.33	2.42 ± 0.57	1.70
Frontal	0.13 ± 0.04	2.93	0.13 ± 0.09	12.99	0.12 ± 0.07	19.61	0.07 ± 0.01	6.17	1.87 ± 1.25	16.81	3.14 ± 0.84	0.61
Temporal	0.13 ± 0.05	3.74	0.16 ± 0.11	14.87	0.14 ± 0.07	17.14	0.06 ± 0.01	5.14	2.57 ± 1.48	16.29	3.19 ± 0.92	0.65
Parietal	0.13 ± 0.04	2.90	0.11 ± 0.06	13.15	0.11 ± 0.05	19.75	0.07 ± 0.01	5.79	1.67 ± 0.78	17.22	3.17 ± 0.86	0.57
Occipital	0.22 ± 0.11	7.43	0.60 ± 0.68	19.86	0.29 ± 0.16	17.79	0.06 ± 0.01	6.23	5.43 ± 3.91	17.60	3.17 ± 0.88	0.98
Cingulate	0.15 ± 0.05	4.81	0.18 ± 0.08	19.60	0.17 ± 0.07	21.93	0.07 ± 0.01	6.36	2.60 ± 0.93	21.40	3.08 ± 0.84	0.77
White matter	0.08 ± 0.02	2.28	0.06 ± 0.02	9.18	0.03 ± 0.02	28.19	0.04 ± 0.01	14.40	0.73 ± 0.26	16.44	2.21 ± 0.61	1.10
Pons	0.08 ± 0.03	4.55	0.15 ± 0.03	8.69	0.02 ± 0.01	21.38	0.02 ± 0.00	20.92	1.07 ± 0.22	10.90	1.16 ± 0.28	4.82
Midbrain	0.09 ± 0.03	3.96	0.16 ± 0.04	7.11	0.02 ± 0.01	15.52	0.01 ± 0.00	17.13	1.35 ± 0.25	8.34	1.29 ± 0.32	4.85

Figures

Figure 1

Concentration of radioactivity and composition of plasma radioactivity in arterial plasma after injection of 18F-T-401 in 7 subjects (a) Time course of concentration of radioactivity in plasma. Data points from 0 to 120 min are shown in the two graphs with different y-axis ranges. (b) Fractions of the parent and radiometabolites of 18F-T-401 in plasma. Parent (black circles) and radiometabolites (metabolite 1: white squares, metabolite 2: white triangles, metabolite 3: white inverted triangles, metabolite 4: white diamonds, metabolite 5: white circles) are plotted against time after injection. Data are shown as mean $\pm$ SD from 7 subjects. (c) Representative radiochromatogram of plasma at 20 min after injection of 18F-T-401. SUV: standardized uptake value

Figure 2

Average time-course of radioactivity after injection of 18F-T-401 (a) Concentration of radioactivity from 4 regions is shown in the frontal lobe (circles), putamen (triangles), white matter (diamonds) and pons (inverse triangles). Data are mean $\pm$ SD from 7 subjects. (b) Representative images in a healthy subject injected with 18F-T-401. Axial PET images were obtained by averaging from 60 to 120 min and were fused with corresponding T1-weighted MR images. SUV, standardized uptake value

Figure 3

Graphical analysis and compartment model of $^{18}\text{F-T401}$ by arterial input function (a) Curve fitting of the 120-min data of $^{18}\text{F-T401}$ by Logan graphical analysis in the frontal lobe (circles), putamen (squares), white matter (diamonds) and pons (inverse triangles) in the same subject. The plots are linear after 20 min (t^*). (b) Representative imaging in a healthy subject injected with $^{18}\text{F-T401}$. Horizontal (left), coronal (middle) and axial (right) PET images of Logan VT were fused with corresponding T1-weighted MR images. (c) Concentrations of radioactivity in the frontal lobe (circles), putamen (squares), white matter (diamonds) and pons (inverse triangles) after injection of $^{18}\text{F-T401}$ fitted with 1TCM (dashed lines) and 2TCM (solid lines). SUV, standardized uptake value 1TCM: one-tissue compartment model, 2TCM: two-tissue compartment model, VT: total distribution volume

Figure 4

Correlations of 2TCM V_T with MA1 (A, left) and Logan V_T (B, right) V_T values with 2TCM were plotted on the x-axis and V_T values with MA1 and Logan graphical analysis were plotted on the y-axis. Each brain region is shown as a black circle. The V_T values estimated by MA1 and Logan graphical analysis are well correlated with the V_T values estimated with 2-TCM ($p < 0.0001$ and $r = 0.99$ for both). MA1: multilinear analysis, 2TCM: two-tissue compartment model, V_T : total distribution volume

Figure 5

Regional V_T values estimated by MA1 Regional V_T values estimated by MA1 are shown. The vertical line indicates standard deviation. MA1: multilinear analysis, V_T : total distribution volume

Figure 6

Time-stability analysis of VT estimation by MA1 Time-stability analysis of MA1 VT values of 18F-T-401 were plotted as a function of data duration truncated from 120 to 30 min. Values are normalized as percentage of terminal value obtained with 120 min data. Dotted lines indicate 100% of the normalized VT value. MA1: multilinear analysis, VT: total distribution volume

Figure 7

Correlation between regional 18F-T-401 VT values and mRNA expression levels for MGLL Average VT values with MA1 in 7 subjects were plotted on the x-axis and normalized expression level (unitless) of MGLL was plotted on the y-axis. Each brain region is shown as a black circle (cerebral cortex, hippocampal formation, basal ganglia, cerebellum, midbrain, pons). The VT values estimated by MA1 and the normalized expression level of MGLL are well correlated (nonparametric $p = 0.0067$ and $r = 0.9286$). MA1: multilinear analysis, VT: total distribution volume

Supplementary Files

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