Human Whole-Body Biodistribution and Dosimetry of a New PET Tracer, [¹¹C]Ketoprofen Methyl Ester, for Imagings of Neuroinflammation

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Introduction: Neuroinflammatory processes play an important role in the pathogenesis of Alzheimer's disease and other brain disorders, and nonsteroidal anti-inflammatory drugs (NSAIDs) are considered therapeutic candidates. As a biomarker of neuroinflammatory processes, ^{11}C -labeled ketoprofen methyl ester (^{11}C]KTP-Me) was designed to allow cerebral penetration of ketoprofen (KTP), an active form of a selective cyclooxygenase-1 inhibitor that acts as an NSAID. Rat neuroinflammation models indicate that ^{11}C]KTP-Me enters the brain and is retained in inflammatory lesions, accumulating in activated microglia. ^{11}C]KTP-Me is washed out from normal tissues, leading to the present first-in-human exploratory study. **Methods:** ^{11}C]KTP-Me was synthesized by rapid C- ^{11}C]methylation of ^{11}C]CH₃I and the corresponding arylacetate precursor, purified with high-performance liquid chromatography, and prepared as an injectable solution including PEG400, providing radiochemical purity of >99% and specific activity of >25 GBq/ μmol at injection. Six young healthy male humans were injected with ^{11}C]KTP-Me and scanned with PET camera to determine the early-phase brain time course, followed by three whole-body scans starting 8, 20, and 40 min post-injection, together with sequential blood sampling and labeled metabolite analysis. **Results:** No adverse effects were observed during PET scanning after ^{11}C]KTP-Me injection. ^{11}C]KTP-Me was rapidly metabolized to ^{11}C -labeled ketoprofen (^{11}C]KTP) within 2-3 min and was gradually cleared from blood. The radioactivity entered the brain with an average peak cortical SUV of 1.5 at 2 min. The cortical activity was gradually washed out. Whole-body images indicated that the urinary bladder was the major excretory pathway. The organ with the highest radiation dose was the urinary bladder (average dose of 41 $\mu\text{Gy/MBq}$, respectively). The mean effective dose was 4.7 $\mu\text{Sv/MBq}$, which was comparable to other ^{11}C -labeled radiopharmaceuticals. **Conclusion:** ^{11}C]KTP-Me demonstrated a favorable dosimetry, biodistribution, and safety profile. ^{11}C]KTP-Me entered the human brain, and the radioactivity was washed out from cerebral tissue. These data warrant further exploratory studies on patients with neuroinflammation.

Key Words: neuroinflammation, ^{11}C]ketoprofen methyl ester, NSAIDs, dosimetry, biodistribution

1. Introduction

Neuroinflammatory processes with microglial activation play an important role in Alzheimer's disease (AD) and other brain disorders such as multiple sclerosis and brain infarction. In the AD brain, *in vitro* experiments have shown increased expression of pro-inflammatory factors such as cytokines and accumulation of activated microglia, suggesting that amyloid- β protein induces neuroinflammatory responses by promoting the production of pro-inflammatory factors from macrophages and/or microglia [1]. Translocator protein is a marker of activated microglia, and [^{11}C]PK11195, [^{11}C]PBR28, and other translocator protein ligands labeled with positron emitters have been used as probes for neuroinflammatory processes in AD and other disorders using positron emission tomography (PET) imaging on animals and humans [2-4].

Cyclooxygenase (COX), an enzyme in the biosynthetic pathway of prostaglandins that plays an important role in inflammatory processes, is considered another potential target for PET imaging of neuroinflammatory processes. According to epidemiological studies, the prevalence of AD is low in patients with long-term consumption of non-steroidal anti-inflammatory drugs (NSAIDs), most of which specifically inhibit COX enzymatic activity [5]. NSAIDs and their derivatives have been labeled with ^{11}C , and their potential as a probe for neuroinflammatory processes was explored. [^{11}C]ketoprofen methyl ester ([^{11}C]KTP-Me) was found to be a promising PET probe based on *in vivo* PET imaging of neuroinflammatory animal models [6].

KTP is a commercially available NSAID and is a selective COX-1 inhibitor. Because permeability of KTP into the brain is poor, [^{11}C]KTP-Me was designed by methyl esterification to allow cerebral penetration of KTP. [^{11}C]KTP-Me enters the brain after intravenous injection and is hydrolyzed to [^{11}C]KTP within the brain, where it likely binds to COX-1 and remains in the brain. Thus, [^{11}C]KTP-Me is expected to be a potential PET probe for neuroinflammatory processes [6, 7].

The present study was designed as the first-in-human study of [^{11}C]KTP-Me to investigate the

safety, radiation dosimetry, pharmacokinetics, whole-body biodistribution, and brain uptake.

2. Materials and methods

2.1 Radiopharmaceutical Preparation

[¹¹C]KTP-Me was synthesized using the synthesizer module CFN-MPS100 (Sumitomo Heavy Industry Co., Tokyo, Japan). Under improved conditions on the basis of the previously reported synthetic protocol [6,7], [¹¹C]CH₃I was reacted with the precursor, (3-benzoylphenyl)acetic acid methyl ester, in the presence of tetra-*n*-butylammonium fluoride as a base in dimethylformamide. The crude solution was purified with high-performance liquid chromatography (HPLC) and prepared for injection. The final solution contained ascorbic acid and 5.9% polyethylene glycol 400 as additives. This synthesis process produced [¹¹C]KTP-Me with a radiochemical purity of 99.89 ± 0.06%, a specific activity of 55.6 ± 14.8 GBq/μmol, and a radiochemical yield of 26.4 ± 7.7%.

2.2 Participants

The study was approved by the ethics committee of the Institute of Biomedical Research and Innovation (IBRI). Six healthy young male volunteers (mean age ± SD, 33.7 ± 9 years) participated in the study. Written informed consent was obtained from all participants.

Upon entry, all volunteers were screened by obtaining their history, physical examination, vital signs (blood pressure, heart rate, and temperature), electrocardiography, and urine and blood tests (whole blood count, sodium, potassium, chloride, urea nitrogen, creatinine, uric acid, total cholesterol, triglyceride, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, γ-glutamyl transpeptidase, creatine kinase, bilirubin, total protein, albumin), and were free of current medical and psychiatric illnesses. These items were also examined as the safety tests as described later. Blood infectious disease tests (antigen screening for hepatitis B and HIV, and antibody screening for syphilis, HIV, and hepatitis C) were also performed as screening tests.

2.3 PET Scans

The participants were administered intravenously with 120-350 MBq of [^{11}C]KTP-Me and underwent a dynamic brain PET scan and sequential whole-body PET scans using an SET-3000 G/X scanner (Shimazu) [8]. In the whole-body mode of this scanner, the bed moved continuously, and the transmission and emission data were acquired simultaneously. The transmission data for the brain dynamic scan were acquired before injection. Because no human data were previously available, the first volunteer was injected with as low as 120.9 MBq of [^{11}C]KTP-Me, and a 2 min dynamic brain scan was performed followed by a quick chest and abdomen scan in the whole-body mode. As the radioactivity distribution showed no difference from that seen in rats that would drastically alter the dosimetry, the other five participants were injected with 292-350 MBq of [^{11}C]KTP-Me, and a brain dynamic PET scan was acquired for 6 min starting injection (12 frames of 10 sec, 4 frames of 30 sec, 2 frames of 60 sec) [7]. In all six participants, whole-body emission scans were performed in the craniocaudal direction from head to thigh starting at 8, 20, and 40 min post-tracer injection, in which the duration and bed speed were 10 min at 2 mm/sec, 16 min at 1.2 mm/sec, and 25 min at 0.8 mm/sec, respectively. Sequential venous blood sampling of 7 mL was performed before injection and 1, 1.5, 2, 3, 4.5, 6, 18, and 50 min after tracer injection for measurement of the whole-blood and plasma radioactivity concentration with a gamma counter (Auto Well Gamma System, ARC-400, Aloka Co., Tokyo, Japan). Urine was collected from all volunteers at about 60 min post injection to measure the excreted activity. The safety tests, blood, urine, vital signs, and electrocardiography were obtained before injection and between 120 and 150 min post-injection.

2.4 Image Analysis and Dosimetry

PET data were reconstructed using the Dynamic Row-Action Maximum-likelihood Algorithm (DRAMA) and Fourier rebinning (FORE). Reconstructions of brain dynamic emission scans and whole-body static emission scans were performed with 1 iteration and 128 filter cycles, and with

4 iterations and 0 filter cycles, respectively. The images were corrected for attenuation using the transmission data collected over the same region as the emission data. The images were calibrated to Bq/mL and analyzed using PMOD software, version 3.4 (PMOD Technologies). On the brain dynamic images, circular regions of interest (ROIs) 10 mm in diameter were drawn over the frontal, temporal, parietal, and occipital cortices, and the average time activity curve of the cortex was obtained. The activity of cerebral cortex was expressed as standard uptake value (SUV), which is calculated as: mean ROI radioactivity (Bq/mL) / injected activity per body weight (Bq/g). On the whole-body images, ROIs were placed over major organs (brain, lungs, mediastinum, liver, spleen, gallbladder, kidneys, bladder, and ureter) on the whole-body images. A ROI was not drawn over the red bone marrow because no substantial tracer accumulation was noted. The activity in each source organ and the remainders were divided by the injection activity to obtain the uptake as a percent of the injected activity. The residence time of each source organ was calculated from the percent injection activity of each source organ and the time course information by fitting to an exponential curve using OLINDA 1.0/EXM software (Vanderbilt University [9]). The residence time of the urinary bladder was determined by applying the 2-hour interval voiding bladder model of the OLINDA to the bladder image data by fitting an exponential curve together with the drug information of KTP based on the finding in the present study that most of the blood radioactivity was in the form of KTP 3 min post-injection. Dosimetry was determined by entering the average residence time of each source organ into OLINDA 1.0/EXM software using the adult male phantom.

2.5 Analysis of Metabolites in Blood Samples

Using a modification of the previously published preparation protocol [4], a 2-fold volume of acetonitrile was added to a plasma aliquot, and the resulting mixture was centrifuged at $2,190 \times g$ for 5 min at 25 °C. The supernatant was evaporated with nitrogen gas and reconstituted with the

HPLC mobile phase. Radioactive components were analyzed using an HPLC system (LC-10AD Shimadzu Co., Kyoto, Japan) with a NaI(Tl) radiation detector (US-2000, Universal Giken, Kanagawa, Japan) to measure intact tracer and its metabolites, and the elution was monitored by UV absorbance at 254 nm. An Atlantis dc18 Prep column (10 × 250 mm; Waters Milford, MA, USA) was used at a flow rate of 8.0 ml mobile phase/min. After injection of 5 ml of the sample, a fast-gradient process was performed as described [4]. The amount of radioactivity associated with intact radiotracer and its metabolites were calculated as a percentage of the total amount of radioactivity.

3. Results

Symptoms, physical examination, vital signs, electrocardiogram, urine tests, and blood tests did not show any significant abnormal findings 120-150 min after injection of [^{11}C]KTP-Me compared with before injection. The whole-blood and plasma radioactivity concentrations reached a peak around 6 min and then decreased gradually (Fig. 1A). In the plasma metabolite analysis, the parent form ([^{11}C]KTP-Me) disappeared in 2-3 min and was replaced by a hydrophilic metabolite, which was identified as [^{11}C]KTP (Fig. 1B). The data suggested that [^{11}C]KTP-Me in plasma was rapidly hydrolyzed to carboxylic acid form.

The early-phase summation image of the brain for 0-2 min post-injection appeared to be a blood flow distribution pattern, indicating that the radioactivity entered the brain based on the blood flow (Fig. 2A). When the brain images were extracted from the three whole-body scans starting 8, 20, and 40 min post-injection and the summation images were created, the late-phase brain images indicated that activity in the cortex was washed out, and higher radioactivity was observed in the venous sinus (Fig. 2B). Whole-brain and cerebral cortex time-activity curves indicated that the radioactivity rapidly entered the brain with an average peak cortical SUV of about 1.5 at 2 min post-injection, and then it was gradually washed out (Fig. 3). The cortical activity was washed out to 81.2% of the peak

at 6 min and further to 41.3% at 60 min.

The whole-body images revealed transient uptake by the lungs and liver and adsorption to the injected vein. Blood pool activity remained until the late phase. The major excretory pathway of radioactivity was the urinary bladder, with 23.7% of injected activity excreted by 60 min. Excretion of radioactivity via the biliary system was also observed but was minimal (Fig. 4). The organs with the highest absorbed dose ($\mu\text{Gy}/\text{MBq}$) were the urinary bladder ($41 \mu\text{Gy}/\text{MBq}$), the kidneys ($23 \mu\text{Gy}/\text{MBq}$), and the lungs ($13 \mu\text{Gy}/\text{MBq}$). The average effective dose was $4.7 \mu\text{Sv}/\text{MBq}$ (Table 1).

4. Discussion

In this report, we present the results of the first-in-human study with [^{11}C]KTP-Me, a potential new PET tracer for imaging neuroinflammatory processes.

The early brain images and the cortical time activity curve indicated that [^{11}C]KTP-Me entered the brain based on the blood flow and was washed out, which were expected according to the design of the tracer. Higher accumulation in the cerebral cortex than white matter is similar to the results observed in the normal mice and rats *ex vivo* and *in vivo* studies. The peak cortical SUV was rather low (1.5) and was lower than that of [^{11}C]PiB, probably due to temporary retention in the lungs and rapid metabolism in the plasma [10]. However, the peak cortical SUV of this level was considered sufficient for it to be positive with visual interpretation if a retention mechanism exists.

The late-phase brain images revealed much higher blood pool activity than activity in the brain tissue, suggesting that the radionuclide in the late-phase plasma does not enter the brain. This observation is in agreement with the finding that most of the plasma radioactivity after 3 min post-injection is in the form of [^{11}C]KTP, which does not enter the brain. Therefore, the plasma metabolite does not hamper image interpretation or quantification.

The effective dose of [^{11}C]KTP-Me was $4.7 \mu\text{Sv}/\text{MBq}$, which is similar to that of other ^{11}C -

labeled PET tracers. The doses to organs of excretion (kidney and bladder) were relatively high and reflected the fact that the major excretory route of the radioactivity for this PET tracer is the urinary tract. In addition, mild visualization of the bile duct was observed in the images obtained at 20 and 40 min post-injection with mild accumulation in the liver, suggesting minor excretion to the bile duct.

In the present study, [^{11}C]KTP-Me was washed out from the brain in normal volunteers in which no neuroinflammation or no accumulation of activated microglia with COX-1 overexpression were present. As indicated by immunohistochemical study of brain biopsy specimens from patients with Alzheimer's disease, showing increases in COX-1 positive microglia in association with A β plaques [11], if neuroinflammatory processes are present and COX-1 is overexpressed, retention of [^{11}C]KTP-Me may be observed. Since inhibitory activity of KTP on COX-1 (IC_{50} 47 nM) is slightly high as compared with general same type of PET probes, [^{11}C]KTP-Me may not be able to visualize human neuroinflammation as contrasted to the normal brain. However, in the rats study, neuroinflammation resulted in increases of COX-1 activity in activated microglia and also caused significant accumulation of [^{11}C]KTP-Me in inflammatory area, suggesting that neuroinflammation in human brain could be detected by this probe when enough amounts of COX-1 activity of activated microglia are present.

The safety, radiation dosimetry, pharmacokinetics, and whole-body biodistribution data obtained in the present study allow us to proceed to the next step of this study by examining patients with AD and mild cognitive impairment (MCI) or other disorders in which neuroinflammatory processes may be present.

5. Conclusion

[^{11}C]KTP-Me is a safe PET tracer with low radiation exposure that is comparable to that of other ^{11}C -labeled PET tracers. [^{11}C]KTP-Me entered the human brain, whereas its labeled metabolite

did not, and the activity was washed out from cerebral tissue in the absence of neuroinflammation. The results of this study warrant further exploratory studies using this tracer in patients who are considered to have neuroinflammatory processes.

Acknowledgments

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Figure Legend

Fig. 1. Time course of radioactivity concentration in whole blood and plasma following intravenous injection of [^{11}C]KTP-Me in six normal volunteers (Mean $\pm$ SD) (A). Radio-HPLC charts of plasma following injection of [^{11}C]KTP-Me in a normal volunteer (B).

Fig. 2. Brain summation image during 2 minutes after injection of [^{11}C]KTP-Me in a young normal volunteer (A). Late-phase brain summation image created from three whole-body scans (8, 20, and 40 minutes post-injection) (B). The reference CT images of the slice level corresponding to the PET images(C)

Fig. 3. Time-activity curves of whole brain (A) and cerebral cortex (B) after injection of [^{11}C]KTP-Me in six young normal volunteers (Mean $\pm$ SD).

Fig. 4. Biodistribution of radioactivity in a healthy volunteer obtained with whole-body scans starting 8, 20, and 40 minutes after injection of [^{11}C]KTP-Me. Images are decay-corrected. Arrows ($\leftarrow$) indicate biliary excretion.

Table 1

Radiation dosimetry data for [^{11}C]KTP-Me: Organ doses and effective dose in six healthy volunteers

Figure1

Figure2

Figure3

Figure4

Table1

Target organs	Organ dose($\mu\text{Gy}/\text{MBq}$)
Adrenals	1.39 $\pm$ 0.17
Brain	1.62 $\pm$ 0.35
Breasts	0.59 $\pm$ 0.09
Gallbladder Wall	5.02 $\pm$ 3.20
LLI Wall	0.95 $\pm$ 0.10
Small Intestine	0.64 $\pm$ 0.07
Stomach Wall	0.64 $\pm$ 0.07
ULI Wall	0.64 $\pm$ 0.07
Heart Wall	7.78 $\pm$ 2.19
Kidneys	22.6 $\pm$ 7.70
Liver	6.19 $\pm$ 0.68
Lungs	13.5 $\pm$ 3.75
Muscle	0.55 $\pm$ 0.04
Ovaries	0.92 $\pm$ 0.09
Pancreas	1.15 $\pm$ 0.14
Red Marrow	0.62 $\pm$ 0.05
Osteogenic Cells	0.41 $\pm$ 0.03
Skin	0.26 $\pm$ 0.02
Spleen	3.64 $\pm$ 1.55
Testes	0.60 $\pm$ 0.06
Thymus	0.96 $\pm$ 0.15
Thyroid	0.21 $\pm$ 0.04
Urinary Bladder Wall	41.6 $\pm$ 4.47
Uterus	1.99 $\pm$ 0.21
Total Body	1.02 $\pm$ 0.08

Data are mean $\pm$ SD. Effective dose is 4.7 $\pm$ 0.35 $\mu\text{Sv}/\text{MBq}$

LLI, Lower Large Intestine; ULI, Upper Large Intestine