ARTICLE IN PRESS

Nuclear Medicine and Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

Identification of a major radiometabolite of [^{11}C]PBB3

Hiroki Hashimoto [a], Kazunori Kawamura [a,*], Makoto Takei [a], Nobuyuki Igarashi [a,b], Tomoya Fujishiro [a,b], Satoshi Shiomi [a,b], Ryuji Watanabe [a,b], Masatoshi Muto [a,b], Kenji Furutsuka [a,c], Takehito Ito [a,c], Tomoteru Yamasaki [a], Joji Yui [a], Kazuyoshi Nemoto [a], Yasuyuki Kimura [a], Makoto Higuchi [a], Ming-Rong Zhang [a]

[a] *Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan*
[b] *Tokyo Nuclear Services Co., Ltd., Tokyo, Japan*
[c] *SHI Accelerator Service Ltd., Tokyo, Japan*

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form 14 August 2015
Accepted 24 August 2015
Available online xxxx

Keywords:
[^{11}C]PBB3
tau probe
PET
radiometabolite
sulfotransferase
cytochrome P450

ABSTRACT

Introduction: [^{11}C]PBB3 is a clinically used positron emission tomography (PET) probe for in vivo imaging of tau pathology in the brain. Our previous study showed that [^{11}C]PBB3 was rapidly decomposed to a polar radiometabolite in the plasma of mice. For the pharmacokinetic evaluation of [^{11}C]PBB3 it is important to elucidate the characteristics of radiometabolites. In this study, we identified the chemical structure of a major radiometabolite of [^{11}C]PBB3 and proposed the metabolic pathway of [^{11}C]PBB3.

Methods: Carrier-added [^{11}C]PBB3 was injected into a mouse for in vivo metabolite analysis. The chemical structure of a major radiometabolite was identified using LC–MS. Mouse and human liver microsomes and liver S9 samples were incubated with [^{11}C]PBB3 in vitro. In silico prediction software was used to assist in the determination of the metabolite and metabolic pathway of [^{11}C]PBB3.

Results: In vivo analysis showed that the molecular weight of a major radiometabolite of [^{11}C]PBB3, which was called as [^{11}C]M2, was m/z 390 [M+H^{+}]. In vitro analysis assisted by in silico prediction showed that [^{11}C]M2, which was not generated by cytochrome P450 enzymes (CYPs), was generated by sulfated conjugation mediated by a sulfotransferase.

Conclusion: The major radiometabolite, [^{11}C]M2, was identified as a sulfated conjugate of [^{11}C]PBB3. [^{11}C]PBB3 was metabolized mainly by a sulfotransferase and subsidiarily by CYPs.

1. Introduction

A class of phenyl- and pyridyl-butadienyl-benzothiazoles (PBBs) has been developed by our research group for use as potential tau imaging probes [1,2]. Structurally, compounds in the PBB series are analogs of a fluorescent amyloid dye in which the two aromatic moieties are spaced with an all-trans butadiene bridge (Fig. 1) [1]. Among these PBB compounds, 2-[(1E,3E)-4-[6-([^{11}C]methylamino)pyridin-3-yl] buta-1,3-dien-1-yl]-1,3-benzothiazol-6-ol ([^{11}C]PBB3, Fig. 1) was found to be the best candidate, with a high affinity for tau aggregates in the nanomolar range ($K_d = 2.55$ nM) and selectivity for tau over Aβ deposits (50-folds) [1].

[^{11}C]PBB3 was radiosynthesized as a candidate PET probe for tau protein aggregates [3], which are the hallmark pathologies of Alzheimer disease (AD) and a variety of other neurodegenerative disorders [4]. The utility of [^{11}C]PBB3 in PET imaging of tau pathology in living brains was initially evaluated in tau transgenic mouse models [1]. [^{11}C]PBB3 yielded superior signals with less nonspecific binding in the brainstem of PS19 tau transgenic mice compared with a β-amyloid PET probe, [^{11}C]PIB. According to these preclinical results, we applied [^{11}C]PBB3 to the clinical PET studies of normal older-adult controls and patients with AD and other neurodegenerative tauopathies [1]. [^{11}C]PBB3 was shown to capture tau lesions in these patients, and it was noteworthy that the high-level retention of [^{11}C]PBB3 in the AD hippocampus, wherein tau pathology is enriched, contrasted sharply with that of [^{11}C]PIB [1].

Our previous study also revealed the presence of a polar radioactive metabolite of [^{11}C]PBB3 in mouse and human plasma [1,3]. The percentage of unmetabolized [^{11}C]PBB3 was less than 2% as early as 1 min after injection in mice, although the metabolic conversion was relatively slow in human (<8% at 3 min after injection) [3]. These results may explain the lower uptake of [^{11}C]PBB3 (1.92% ID/g at 1 min after injection) by the brain compared with that of other radioprobes applied to clinical PET imaging of tau deposits, such as [^{18}F]T807 [5,6] and [^{18}F]THK compounds [7,8]. The same radiometabolite of [^{11}C]PBB3 was also found in the mouse brain after radioprobe injection, potentially hampering a simplified image analysis. Thus, the determination of the metabolic pathway for [^{11}C]PBB3 and the identification of the chemical structure of the metabolites are required to further assess the clinical utility of [^{11}C]PBB3 and to design new PBB analogs that are more biostable and that enter the brain more efficiently than the parent compound.

---

* Corresponding author at: Molecular Probe Program, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan. Tel.: +81 43 382 3707; fax: +81 43 206 3261.
E-mail address: kawamur@nirs.go.jp (K. Kawamura).

http://dx.doi.org/10.1016/j.nucmedbio.2015.08.006
0969-8051/© 2015 Elsevier Inc. All rights reserved.

Please cite this article as: Hashimoto H., et al, Identification of a major radiometabolite of [^{11}C]PBB3, Nucl Med Biol (2015), http://dx.doi.org/10.1016/j.nucmedbio.2015.08.006

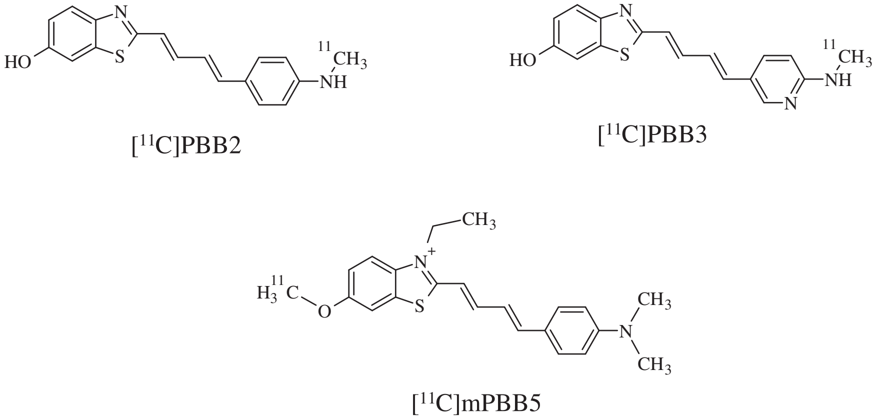

Fig. 1. Chemical structures of [^{11}C]PBB3 and ^{11}C-labeled PBB analogs.

In this study, we identified the chemical structure of a major radiometabolite of [^{11}C]PBB3 using LC–MS and proposed the metabolic pathway of [^{11}C]PBB3 by in vivo, in vitro, and in silico studies.

2. Materials and methods

2.1. General

PBB3 and its precursor for radiosynthesis were purchased from the NARD Institute (Kobe, Japan). Commercially available reagents and organic solvents (Sigma-Aldrich, St Louis, MO; Wako Pure Chem., Osaka, Japan) were used without further purification. Carbon-11 was produced by ^{14}N(p, α)^{11}C nuclear reaction using a CYPRIS HM18 cyclotron (Sumitomo Heavy Industries, Tokyo, Japan). Effluent radioactivity was monitored using a NaI (Tl) scintillation detector system (Gabi Star PET, Raytest, Straubenhardt, Germany). If not otherwise stated, radioactivity was determined using an IGC-3R or IGC-7 curiemeter (Aloka, Tokyo, Japan).

A male inbred strain of laboratory mice (ddY) were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were maintained and handled in accordance with the recommendations of the U.S. National Institutes of Health and the guidelines of the National Institute of Radiological Sciences. Animal experiments were approved by the Animal Ethics Committee of the National Institute of Radiological Sciences. The mice were fed ad libitum.

The sample of metabolite analysis was analyzed using an HPLC system equipped with a radioactivity detector (radio-HPLC) [9]. The HPLC system and measurement conditions were as follows: pump, PU-2089 plus (Jasco, Tokyo, Japan); UV detector, UV-2075 (Jasco); radioactivity detector (OKEN S-2493A, Ohyo Koken Kogyo, Tokyo, Japan); precolumn, XBridge Prep C18 Guard Cartridge (5 μm, 10 i.d. × 10 mm, Waters, Milford, MA); main column, XBridge OST C18 (2.5 μm, 10 i.d. × 50 mm, Waters); mobile phase, 90% aqueous acetonitrile/0.02 mol/L sodium phosphate buffer (pH 7.0) [30/70 (0–4 min), 30/70 to 70/30 (linear gradient, 4–7 min) vol/vol]; flow rate, 8.0 mL/min.

Also, the sample of metabolite analysis was analyzed using LC–MS. The LC–MS system was as follows: HPLC system (Agilent, Santa Clara, CA); MS detector, 4000QTRAP (AB Sciex, Framingham, MA); column, XBridge Shield RP18 (2.5 μm, 3.0 i.d. × 50 mm, Waters). The LC and MS conditions were as follows: column, XBridge Shield RP18 column (3.0 × 50 mm, 2.5 μm, Waters); mobile phase, 90% aq. acetonitrile and water [10/90 (0–2 min), 10/90 to 70/30 (linear gradient, 2–7 min)]; flow rate, 0.5 mL/min; MS (100–400 *m/z*), enhanced product ion scan mode (collision energy, 25); data acquisition, Analyst software (AB Sciex). Identification of the [^{11}C]PBB3 metabolite was performed by the collision-induced dissociation method using nitrogen gas as the target. The sample was infused into the electrospray probe to tune the mass spectrometer and optimize the source acquisition parameters for the molecular ion *m/z* 310 or 390 (sheath gas with a flow rate of 15 U, source voltage 5.5 kV, capillary voltage 40 V, positive mode).

All procedures were conducted with the fluorescent light switched off to prevent photoisomerization of [^{11}C]PBB3 and radiometabolites. If necessary, a UV-cut flashlight (green light, LED-41VIS525, OptoCode, Tokyo, Japan) was used to monitor these procedures for a short time.

2.2. Radiosynthesis of [^{11}C]PBB3

[^{11}C]PBB3 was synthesized as previously described [3]. In brief, the precursor, 5-((1*E*,3*E*)-4-(6-(*tert*-butyldimethylsilyloxy)benzo[*d*]thiazol-2-yl)buta-1,3-dienyl)pyridin-2-amine, was reacted with [^{11}C]methyl iodide to produce 5-((1*E*,3*E*)-4-(6-(*tert*-butyldimethylsilyloxy)benzo[*d*]thiazol-2-yl)buta-1,3-dienyl)-*N*-2-[^{11}C]methylpyridin-2-amine, followed by deprotection with alkali water to produce [^{11}C]PBB3.

2.3. In vivo metabolite analysis of carrier-added [^{11}C]PBB3 in mouse plasma

A solution of [^{11}C]PBB3 (110 MBq) and unlabeled PBB3 (carrier, 3.2 μmol) was injected into the tail vein of a mouse, and the mouse was sacrificed by cervical dislocation 5 min after injection. Blood samples were obtained and centrifuged at 15,000 rpm for 2 min at 4 °C. The plasma (0.5 mL) was separated and transferred to a tube containing acetonitrile (0.5 mL). The mixture was vortexed and centrifuged at 15,000 rpm for 1 min at room temperature to separate the precipitate from the aqueous phase. The supernatants were analyzed using an radio-HPLC or LC–MS as described above. The fraction of a major radiometabolite of [^{11}C]PBB3 was collected to use as a reference of HPLC analysis for in vitro studies.

2.4. In vitro metabolite analysis of [^{11}C]PBB3

2.4.1. Human and mouse liver microsomes

To investigate the metabolism of [^{11}C]PBB3 mediated by cytochrome P450 enzymes (CYPs), we performed an in vitro metabolite analysis of [^{11}C]PBB3 using human or mouse liver microsomes and an NADPH regeneration system as an activator of oxidative reactions by CYPs, as previously described, with minor modifications [10,11]. Pooled human liver microsomes (HLM) and pooled male mouse liver microsomes (MLM) were purchased from Corning (Corning, NY). The reaction mixtures contained 50 μL of HLM or MLM (protein content 20 mg/mL), 50 μL of NADPH regenerating system solution A (31 mmol/L NADP$^+$, 66 mmol/L glucose-6-phosphate, and 66 mmol/L $MgCl_2$ in H_2O; Corning), 10 μL of NADPH regenerating system solution B (40 U/mL glucose-6-phosphate dehydrogenase in 5 mmol/L sodium citrate; Corning), 10 μL of [^{11}C]PBB3 (1.3–6.1 MBq/16–84 pmol), 0.2 mL of 0.1 M Tris buffer (pH 7.5), and 0.64 mL of ultrapure water. The mixtures were incubated in a 37 °C water bath for 60 min. The reaction was

Please cite this article as: Hashimoto H., et al, Identification of a major radiometabolite of [^{11}C]PBB3, Nucl Med Biol (2015), http://dx.doi.org/10.1016/j.nucmedbio.2015.08.006