Nuclear Medicine and Biology 45 (2017) 1–7

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

A useful PET probe [^{11}C]BU99008 with ultra-high specific radioactivity for small animal PET imaging of I_2-imidazoline receptors in the hypothalamus☆

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ARTICLE INFO

Article history:
Received 12 July 2016
Received in revised form 14 October 2016
Accepted 24 October 2016

Keywords:
Ultra-high specific radioactivity
^{11}C
Imidazoline receptors
I_2
BU99008
PET

ABSTRACT

Introduction: A positron emission tomography (PET) probe with ultra-high specific radioactivity (SA) enables measuring high receptor specific binding in brain regions by avoiding mass effect of the PET probe itself. It has been reported that PET probe with ultra-high SA can detect small change caused by endogenous or exogenous ligand. Recently, Kealey et al. developed [^{11}C]BU99008, a more potent PET probe for I_2-imidazoline receptors (I_2Rs) imaging, with a conventional SA (mean 76 GBq/μmol) showed higher specific binding in the brain. Here, to detect small change of specific binding for I_2Rs caused by endogenous or exogenous ligand in an extremely small region, such as hypothalamus in the brain, we synthesized and evaluated [^{11}C]BU99008 with ultra-high SA as a useful PET probe for small-animal PET imaging of I_2Rs.

Methods: [^{11}C]BU99008 was prepared by [^{11}C]methylation of *N*-desmethyl precursor with [^{11}C]methyl iodide. Biodistribution, metabolite analysis, and brain PET studies were conducted in rats.

Results: [^{11}C]BU99008 with ultra-high SA in the range of 5400–16,600 GBq/μmol were successfully synthesized ($n = 7$), and had appropriate radioactivity for *in vivo* study. In the biodistribution study, the mean radioactivity levels in all investigated tissues except for the kidney did not show significant difference between [^{11}C]BU99008 with ultra-high SA and that with conventional SA. In the metabolite analysis, the percentage of unchanged [^{11}C]BU99008 at 30 min after the injection of probes with ultra-high and conventional SA was similar in rat brain and plasma. In the PET study of rats' brain, radioactivity level ($AUC_{30–60\ min}$) in the hypothalamus of rats injected with [^{11}C]BU99008 with ultra-high SA (64 [SUV · min]) was significantly higher than that observed for that with conventional SA (50 [SUV · min]). The specific binding of [^{11}C]BU99008 with ultra-high SA (86% of total binding) for I_2R was higher than that of conventional SA (76% of total binding).

Conclusion: A PET study using [^{11}C]BU99008 with ultra-high SA would thus contribute to the detection of small changes in or small regions with I_2R expression and hence may be useful in elucidating new functions of I_2R.

1. Introduction

A carrier mixed with a positron emission tomography (PET) probe binds to receptors competitively, and hampers the measurement of receptor binding using the PET probe, especially in brain regions with low densities of receptors, because the PET probe-induced receptor occupancy was increased by increasing the mass of PET probe and by underestimating the receptor density [1]. It has been reported that a PET probe with a much lower level of mass, *i.e.*, extremely high specific radioactivity (SA) enabled measuring high receptor binding in brain regions with low receptor densities [1]. Furthermore, it has been reported that PET probe with extremely high specific SA could detect small change caused by endogenous or exogenous ligand, because the extremely high SA avoided mass effect of the PET probe itself [1,2]. We recently developed an automatic synthesis system for preparing ^{11}C-labeled PET probes with an extremely high SA of >2000 GBq/μmol [3]. On applying the PET probes with ultra-high SA, we succeeded in detecting two-affinity binding sites of [^{11}C]raclopride in the striatum and cerebral cortex of rat brain [4]. Additionally, we found that the PET probe [^{11}C]DAC for translocator protein (TSPO, 18 kDa) with ultra-high SA is

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http://dx.doi.org/10.1016/j.nucmedbio.2016.10.005
0969-8051/

a useful and sensitive biomarker for the visualization of early infarction and characterization of TSPO expression, which was slightly elevated in the infarcted brain, by using small-animal PET [5,6]. Furthermore, we reported that the PET probe [^{11}C]ITMM for metabotropic glutamate receptors subtype 1 (mGluR1) with ultra-high SA is a promising tool for elucidating new mechanisms of mGluR1 in the brain [7]. Therefore, the PET probe with ultra-high SA is a useful tool for investigating the receptor functions in low density and small regions of the brain.

Imidazoline receptors (IRs) were investigated based on the observation that the vasodepressor action of centrally administered clonidine could not be mimicked or blocked by adrenergic compounds lacking an imidazoline structure [8]. Later, radiolabeled probe binding studies revealed two subtypes: I_1-imidazoline receptors (I_1Rs, labeled by clonidine) and I_2-imidazoline receptors (I_2Rs, labeled by idazoxan) [8–10]. The I_1Rs are encoded by a non-G-protein-coupled protein called imidazoline receptor antisera-selected protein [11], and possess hypotensive activity [12]. The I_2Rs are located mostly on the outer membrane of mitochondria [13], and I_2R proteins have not been encoded and their functional role remains uncertain. IRs are widely distributed throughout the tissues of various species including humans, and are present in the central and peripheral nervous systems and in various organs such as the kidney, lung, and heart [14]. Additionally, I_2Rs have been linked to several central nervous system disorders [15–24]. Additionally, I_2R has been found in the hypothalamus [25,26], and it has reported that selective I_2R ligands promoted food intake [27,28], which is presumed to be an effect of eating function through the hypothalamus known as a feeding center [29].

The development of selective I_2R PET probes would allow for the characterization of I_2R *in vivo* and its regulation in diseased states. Several PET probes selective for I_2R have been reported [30–35]. Among these, we developed [^{11}C]FTIMD (*Ki* for I_2R, 3.0 nmol/L) as the first *in vivo* imaging agent [32]. Furthermore, [^{11}C]FTIMD with ultra-high SA (mean 4470 GBq/μmol) showed higher specific binding than [^{11}C]FTIMD with conventional SA (about 100 GBq/μmol) in the hypothalamus [36]. However, [^{11}C]FTIMD showed moderate non-specific binding in the brain [32,36,37], which hampered further application study. More recently, Kealey et al. [34] developed [^{11}C]BU99008 (*Ki* for I_2R, 1.4 nmol/L) as a more potent PET probe for I_2R imaging. [^{11}C]BU99008 displayed a relatively high brain penetration and specific binding in the porcine and rhesus brain [34,38]. Recently, [^{11}C]BU99008 is becoming a representative PET probe useful for imaging of I_2Rs. In this study, to detect small change caused by endogenous or exogenous ligand of specific binding for I_2Rs in extremely small regions such as the hypothalamus, we synthesized and evaluated [^{11}C]BU99008 with ultra-high SA as a useful PET probe for small-animal PET imaging of I_2Rs.

2. Materials and methods

2.1. General

BU99008 (Fig. 1) and 2-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*–indole (BU99007, *N*-desmethyl BU99008, Fig. 1) were prepared in our laboratory as described previously [39] with certain modifications. BU224 hydrochloride was purchased from Tocris Bioscience (Bristol, UK). Reagents and organic solvents were commercially available (Sigma-Aldrich, St. Louis, MO, USA; Tokyo Chemical Industry, Tokyo, Japan; Wako Chemical Industries, Osaka, Japan) and were used without further purification.

Preparative high-performance liquid chromatography (HPLC) and analytical HPLC for conventional SA were performed using a Jasco HPLC system (PU-2089 pump; UV-2070 detector; Jasco, Tokyo, Japan). Analytical HPLC for ultra-high SA was performed using a Waters HPLC system (515 pump and 2489 UV detector; Waters, Milford, MA, USA). Effluent radioactivity was monitored using a NaI (Tl) scintillation detector system (OKEN, Tokyo, Japan). Unless otherwise stated, radioactivity was determined using an IGC-3R or IGC-7F Curiemeter (Hitachi, Tokyo, Japan).

Male Sprague Dawley rats (aged, 7 weeks) were purchased from Japan SLC (Shizuoka, Japan). Animals were maintained and handled in accordance with recommendations of the U.S. National Institutes of Health and the guidelines of the National Institute of Radiological Sciences (Chiba, Japan). Animal studies were approved by the Animal Ethics Committee of the National Institute of Radiological Sciences.

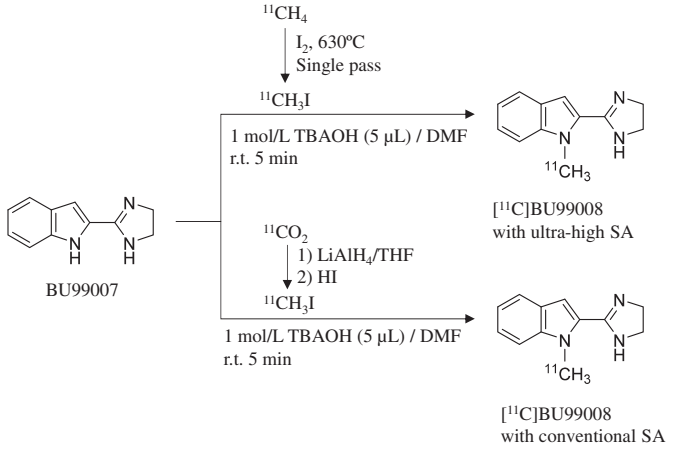

Fig. 1. Radiosynthesis of [^{11}C]BU99008 with ultra-high specific radioactivity (SA) and conventional SA.

2.2. Radiosynthesis of [^{11}C]BU99008

[^{11}C]BU99008 was synthesized according to the method described previously [34] using an automated synthesis system developed in-house [40] (Fig. 1). A solution of BU99007 (1 mg) and 1 mol/L aqueous tetrabutylammonium hydroxide (6 μL) in *N*,*N*-dimethylformamide (DMF, 0.3 mL) was added in a dry septum-equipped vial before radiosynthesis. In the radiosynthesis of [^{11}C]BU99008 with ultra-high SA, [^{11}C]methyl iodide was synthesized by iodination of [^{11}C]methane, which was produced by a cyclotron (CYPRIS HM-18; Sumitomo Heavy Industries, Tokyo, Japan), with iodide using the single-pass method as described previously [3]. In the radiosynthesis of [^{11}C]BU99008 with conventional SA, [^{11}C]methyl iodide was produced by reduction of cyclotron-produced [^{11}C]carbon dioxide with lithium aluminum hydride, followed by iodination with hydriodic acid. Then, the produced [^{11}C]methyl iodide was trapped in the solution of BU99007 in DMF by cooling with air. The reaction mixture was reacted at room temperature for 5 min. After cooling, 1.0 mL of the preparative HPLC eluent was added to the mixture. The solution was applied to the preparative HPLC system mentioned above. Preparative HPLC was performed on a Wakopak Fluofix 120 N column (5 μm, 10 mm internal diameter [i.d.] × 250 mm length; Wako Pure Chemical Industries) using a mixture of methanol and 20 mmol/L sodium phosphate buffer (80:20, vol./vol.) as the mobile phase, at a flow rate of 5 mL/min with an ultraviolet detector at 254 nm and a radioactivity detector. The retention time of [^{11}C]BU99008 and BU99007 was approximately 12 and 17 min, respectively. The HPLC fraction of [^{11}C]BU99008 was collected in a flask to which 25% ascorbic acid (100 μL) and Tween 80 (75 μL) in ethanol (300 μL) had been added before radiosynthesis, and subsequently evaporated to dryness. The residue was dissolved in physiological saline. The final product (20 μL or 100 μL) was analyzed with HPLC on a Capcell Pak C18 UG 80 column (5 μm, 4.6 mm i.d. × 250 mm length; Shiseido, Tokyo, Japan) using a mixture of acetonitrile, water, and triethylamine (50:50:0.01, vol./vol./vol.) as the mobile phase at a flow rate of 1.5 mL/min with an ultraviolet detector at 300 nm and a radioactivity detector. The retention time was 4.6 min.

2.3. Biodistribution study in rats

[^{11}C]BU99008 (ultra-high SA: 10 MBq/1.0 pmol or conventional SA: 10 MBq/150 pmol) was intravenously injected into rats (aged,