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Binding potential of (*E*)-[^{11}C]ABP688 to metabotropic glutamate receptor subtype 5 is decreased by the inclusion of its ^{11}C-labelled *Z*-isomer

Kazunori Kawamura [a,*], Tomoteru Yamasaki [a], Katsushi Kumata [a], Kenji Furutsuka [a,b], Makoto Takei [a,c], Hidekatsu Wakizaka [d], Masayuki Fujinaga [a], Kaori Kariya [a], Joji Yui [a], Akiko Hatori [a], Lin Xie [a], Yoko Shimoda [a], Hiroki Hashimoto [a], Kazutaka Hayashi [e], Ming-Rong Zhang [a]

[a] *Molecular Probe Program, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba 263–8555, Japan*
[b] *SHI Accelerator Service Ltd., Tokyo 141–0032, Japan*
[c] *Tokyo Nuclear Services Co., Ltd., Tokyo 110–0016, Japan*
[d] *Biophysics Program, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba 263–8555, Japan*
[e] *Advanced Molecular Imaging Center, Oita University, Yufu879–5593, Japan*

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ABSTRACT

Introduction: [^{11}C]ABP688 is a promising positron emission tomography (PET) ligand for imaging of metabotropic glutamate receptor subtype 5 (mGlu5 receptor). Of the two geometric isomers of ABP688, (*E*)-ABP688 has a greater affinity towards mGlu5 receptors than (*Z*)-ABP688. Therefore, a high ratio of *E*-isomer is required when using [^{11}C]ABP688 as a PET probe for imaging and quantification of mGlu5 receptors. The aim of this study was to evaluate the effect (*Z*)-[^{11}C]ABP688 on the synthesis of [^{11}C]ABP688 to be used for binding (*E*)-[^{11}C]ABP688 in the brain.

Methods: We synthesized and separated (*E*)- and (*Z*)-[^{11}C]ABP688 by purification using an improved preparative high-performance liquid chromatography (HPLC) method equipped with a COSMOSIL Cholester column. We performed an *in vitro* binding assay in rat brain homogenates and PET studies of the rat brains using (*E*)- and (*Z*)-[^{11}C]ABP688.

Results: (*E*)- and (*Z*)-[^{11}C]ABP688 were successfully obtained with suitable radioactivity for application. In the *in vitro* assay, the K_d value of (*E*)-[^{11}C]ABP688 (5.7 nmol/L) was higher than that of (*Z*)-[^{11}C]ABP688 (140 nmol/L). In the PET study of the rat brain, high radioactivity after injection of (*E*)-[^{11}C]ABP688 was observed in regions rich in mGlu5 receptors such as the striatum and hippocampus. In contrast, after injection of (*Z*)-[^{11}C]ABP688, radioactivity did not accumulate in the brain. Furthermore, BP_{ND} in the striatum and hippocampus was highly correlated ($R^2 = 0.99$) with the percentage of (*E*)-[^{11}C]ABP688 of the total radioactivity of (*E*)- and (*Z*)-[^{11}C]ABP688 in the injection.

Conclusion: We demonstrated that including (*Z*)-[^{11}C]ABP688 in the [^{11}C]ABP688 injection can decrease BP_{ND} in regions rich in mGlu5 receptors. Routine production of (*E*)-[^{11}C]ABP688 will be helpful for imaging and quantification of mGlu5 receptors in clinical studies.

1. Introduction

3-(6-Methylpyridin-2-ylethynyl)-cyclohex-2-enone-*O*-methyloxime (ABP688) is a highly selective allosteric antagonist of the metabotropic glutamate receptor subtype 5 (mGlu5 receptor). Initial studies have demonstrated high specificity of this compound for mGlu5 receptors *in vivo* [1,2]. Dysfunction of mGlu5 receptors has been implicated in disorders such as schizophrenia [3,4], psychostimulant addiction [5,6], Parkinson's disease [7–9], attention-deficit and hyperactivity disorders [10], and degenerative and dysplastic diseases of the central nervous system [11]. As a positron emission tomography (PET) probe for the imaging of mGlu5 receptors, [^{11}C]ABP688 has been developed [1] and evaluated for the quantification of mGlu5 receptors in primates [12,13] and humans [14–21]. [^{11}C]ABP688 may be a valuable tool for developing novel therapeutics to combat these disorders by establishing *in vivo* drug occupancy.

Of the two geometrical isomers of ABP688 [1,2], (*E*)-ABP688 has greater potency for mGlu5 receptors than (*Z*)-ABP688, but the affinity of the *Z*-isomer has not been described [1,2]. Ametamey et al. optimized the synthesis of [^{11}C]ABP688 to achieve an *E*- to *Z*-isomer (Fig. 1) ratio of >10:1 by preheating the sodium salt of the precursor to 90°C and then adding [^{11}C]methyl iodide at this temperature [1]. Additionally, another study improved the radiosynthesis of [^{3}H]ABP688 to obtain an *E*- to *Z*-isomer ratio of 22:1 [22]. Because of the higher potency of the *E*-isomer, a high ratio of radiolabeled (*E*)-

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

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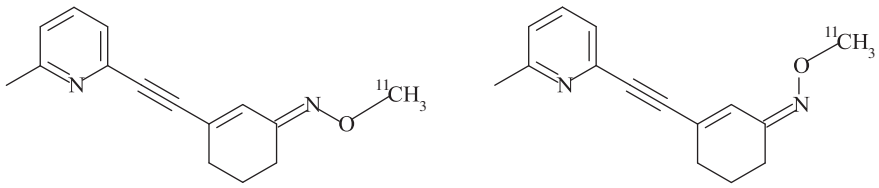

(*E*)-[^{11}C]ABP688 (*Z*)-[^{11}C]ABP688

Fig. 1. Chemical structures of (*E*)- and (*Z*)-[^{11}C]ABP688.

ABP688 should be used in the probe for quantifying mGlu5 receptors. In a recent report, quantitative analysis using [^{11}C]ABP688 showed a significant difference in the binding potential in human brains during test-retest evaluations [20]. However, another study group did not observe a significant difference in binding potentials during test-retest evaluations for [^{11}C]ABP688 in baboons [13]. This instability of binding potential during test-retest evaluations using [^{11}C]ABP688 has not been conclusively demonstrated [20,23]. Further experiments are required to explain differences in test-retest variability in [^{11}C]ABP688.

Thus, we examined whether inclusion of the *Z*-isomer during the synthesis of [^{11}C]ABP688 would cause a decrease in the binding of (*E*)-[^{11}C]ABP688. To evaluate the effect of including (*Z*)-[^{11}C]ABP688, we synthesized and purified radiochemically pure (*E*)- and (*Z*)-[^{11}C]ABP688 and compared their *in vitro* and *in vivo* characteristics.

2. Materials and methods

2.1. General

Desmethyl-ABP688 and ABP688 were synthesized at the NARD institute (Kobe, Japan) according to previously described methods [1,2]. Separation of (*E*)- and (*Z*)-ABP688 was performed using preparative high-performance liquid chromatography (HPLC) with a shimadzu Nexera HPLC system (Shimadzu, Kyoto, Japan). The preparative HPLC conditions were as follows: column, Unison UK C-18 (5 μm, 10 mm internal diameter [i.d.] × 100 mm length; Imtakt, Kyoto, Japan); mobile phase, a mixture of acetonitrile and water (50:50, vol./vol.); flow rate, 3.2 mL/min; ultraviolet detector, 310 nm (local maximum of the ultraviolet-absorbance of ABP688). The retention times of *E*- and *Z*-isomers were approximately 9 min and 8 min, respectively. The identity of the *E*- or *Z*-isomer was confirmed by a proton nuclear magnetic resonance (^{1}H NMR) in UBE Scientific Analysis Laboratory (Yamaguchi, Japan). Analytical data for the *E*- or *Z*-isomers agreed with that reported previously [1,2].

Commercially available reagents and organic solvents were used without further purification. Preparative HPLC was performed using a Jasco HPLC system (Jasco, Tokyo, Japan). Analytical HPLC was performed using a Waters HPLC system (Waters, Milford, MA, USA). Effluent radioactivity was monitored using a NaI(Tl) scintillation detector system. If not otherwise stated, radioactivity was determined using an IGC-3R Curiemeter (Aloka, Tokyo, Japan).

Male Sprague-Dawley (SD) rats were purchased from Japan SLC Inc. (Shizuoka, Japan). 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 (Chiba, Japan). Animal experiments were approved by the Animal Ethics Committee of the National Institute of Radiological Sciences.

2.2. Radiosynthesis of (*E*)- or (*Z*)-[^{11}C]ABP688

(*E*)- or (*Z*)-[^{11}C]ABP688 (Fig. 1) was synthesized as previously described to synthesize [^{11}C]ABP688 [1] with the following modifications. [^{11}C]Methyl iodide was synthesized from [^{11}C]carbon dioxide produced using a cyclotron (CYPRIS HM-18; Sumitomo Heavy Industries, Tokyo, Japan) with an in-house automated synthesis system [24]. [^{11}C]Methyl iodide was trapped in a solution of *N*,*N*-dimethylformamide (DMF, 0.2 mL) containing desmethyl-ABP688 (0.2 mg) and sodium hydride (0.2 mg) by cooling. The reaction mixture was heated at 100°C for 4 min. After cooling, 1.0 mL of the preparative HPLC eluent was added to the mixture. Solution components were separated by preparative HPLC. Preparative HPLC was performed using a COSMOSIL Cholester column (5 μm, 10 mm i.d. × 250 mm length; Nakalai Tesque, Kyoto, Japan), a mixture of acetonitrile and water (50:50, vol./vol.) as the mobile phase at a flow rate of 7 mL/min, an ultraviolet detector at 310 nm, and a radioactivity detector. Retention times of desmethyl-ABP688, (*Z*)-[^{11}C]ABP688 and (*E*)-[^{11}C]ABP688 were approximately 4.2 min, 8.5 min, and 10.4 min, respectively (Fig. 2). The HPLC fraction of (*E*)- or (*Z*)-[^{11}C]ABP688 was collected in a flask into which 25% ascorbic acid (0.1 mL) and Tween-80 (0.075 mL) in ethanol (0.3 mL) had been added before radiosynthesis. These fractions were then evaporated to dryness. The residue was dissolved in physiological saline and sterilized with a Millex-GS filter (Millipore, Billerica, MA, USA). The final product was analyzed by HPLC using an XBridge Shield RP18 column (2.5 μm, 3.0 mm i.d. × 50 mm length; Waters). A mixture of 90% acetonitrile, 100 mmol/L ammonium phosphate buffer (pH 2.0) containing 5 mmol/L sodium 1-octanesulfonate, and 50 mmol/L ammonium phosphate buffer (pH 9.3) (44:28:28, vol./vol./vol.) was used as the mobile phase at a flow rate of 1.0 mL/min. An ultraviolet detector at 310 nm and a radioactivity detector were employed. Retention times of (*Z*)- and (*E*)-ABP688 were 2.1 min and 2.6 min, respectively (Fig. 3).

2.3. In vitro binding assay

SD rats were sacrificed by decapitation under anesthesia. Whole brains were rapidly removed and homogenized in 10 volumes of 50

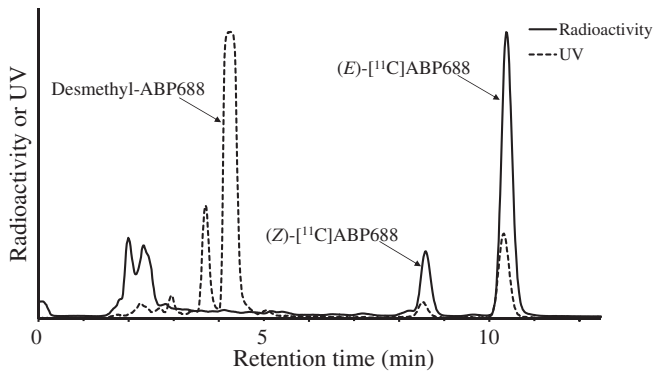

Fig. 2. Radio-HPLC purification of (*E*)-[^{11}C]ABP688. Preparative HPLC conditions were as follows: column, COSMOSIL Cholester (5 μm, 10 mm × 250 mm); mobile phase, a mixture of acetonitrile and water (50:50, vol./vol.); flow rate, 7 mL/min; ultraviolet detector, 310 nm.