Evaluation of iodinated and brominated $[\text{I}]^{11}$C-styrylxanthine derivatives as *in vivo* radioligands mapping adenosine A$_{2A}$ receptor in the central nervous system

Kiichi ISHIWATA,* Junichi SHIMADA,** Wei-Fang WANG,*,*** Hiroyuki HARAKAWA,** Shin-ichi ISHI,* Motohiro KIYOSAWA,*** Fumio SUZUKI** and Michio SENDA*

*Positron Medical Center, Tokyo Metropolitan Institute of Gerontology
**Drug Discovery Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Company
***Tokyo Medical and Dental University

*In vivo* assessment of the adenosine A$_{2A}$ receptors localized in the striatum by PET or SPECT offers us a new diagnostic tool for neurological disorders. In the present study, we evaluated the potential of iodinated and brominated styrylxanthine derivatives labeled with $^{11}$C as an *in vivo* probe. [7-Methyl-$^{11}$C]-(E)-3,7-dimethyl-8-(3-iodostyryl)-1-propargylxanthine ([$^{11}$C]JS-DMPX) and [7-methyl-$^{11}$C]-(E)-8-(3-bromostyryl)-3,7-dimethyl-1-propargylxanthine ([$^{11}$C]BS-DMPX) were prepared by the $^{11}$C-methylation of corresponding 7-demethyl derivatives. *An in vitro* membrane binding study showed a high affinity (Ki values) of the two ligands for A$_{2A}$ receptor: 8.9 nM for IS-DMPX and 7.7 nM for BS-DMPX, and a high A$_{2A}$/A$_{1}$ selectivity: >1100 for IS-DMPX and 300 for BS-DMPX. In mice, [$^{11}$C]IS-DMPX and [$^{11}$C]BS-DMPX were taken up slightly more in the striatum than in the reference regions such as the cortex and cerebellum. The uptake ratios of striatum to cortex and striatum to cerebellum gradually increased but were very small: 1.6–1.7 for the striatum-to-cortex ratio and 1.2 for the striatum-to-cerebellum ratio at 60 min postinjection. The uptake by these three regions was reduced by co-injection of an excess amount of carrier or an A$_{2A}$ antagonist KF17837, but not by an A$_{1}$ antagonist KF15372. The blocking effects in the three regions were greater for [$^{11}$C]BS-DMPX (32–57%) than for [$^{11}$C]IS-DMPX (6–29%). *Ex vivo* autoradiography confirmed that the two ligands were slightly concentrated in the striatum. [$^{11}$C]BS-DMPX showed more selective affinity for adenosine A$_{2A}$ receptors than [$^{11}$C]IS-DMPX, but these results have shown that the two tracers were not suitable as *in vivo* ligands because of low selectivity for the striatal A$_{2A}$ receptors and a high nonspecific binding.

**Key words:** halogenated xanthine, adenosine A$_{2A}$ receptor, PET, SPECT

INTRODUCTION

ADESOSINE is an endogenous modulator of a number of physiological functions in the central nervous system (CNS) as well as in peripheral organs. Recent advances in molecular biology and pharmacology have demonstrated the presence of at least four subtypes i.e., A$_{1}$, A$_{2A}$, A$_{2B}$, and A$_{3}$ receptors. In the CNS, adenosine A$_{1}$ receptors

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For reprint contact: Kiichi Ishiwata, Ph.D., 1–1 Naka-cho, Itabashi, Tokyo 173-0022, JAPAN.
E-mail: ishiwata@pet.tmig.or.jp

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Original Article 247
found in the hippocampus of patients with Alzheimer’s disease\textsuperscript{13,14} and in temporal lobe epilepsy.\textsuperscript{15} In patients with Huntington’s chorea with a selective degeneration of the striatopallidal neurons, adenosine A2A receptor density is significantly reduced in the striatum, but it is not significantly affected in patients with Parkinson’s disease characterized by a selective degeneration of nigrostriatal dopamine neurons.\textsuperscript{16}

Positron emission tomography (PET) assessment of the adenosine receptor system probably offers us a new diagnostic tool for several neurological disorders. For this purpose, we have proposed several PET ligands for the two adenosine receptor subtypes.\textsuperscript{17–24} All ligands were developed based on the findings by Suzuki, Shimada and co-workers showing that xanthine derivatives have a high and selective affinity for adenosine A1 or A2A receptors.\textsuperscript{25,26} [7-Methyl-\({}^{11}\text{C}\)](E)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine ([\(1^{11}\text{C}\)]KF18446) had promising properties as a PET ligand, although [7-methyl-\(1^{11}\text{C}\)-(E)]-1,3-dipropyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthine ([\(1^{11}\text{C}\)]KF17837) had the highest affinity. As for the xanthine-type A2A receptor ligands, the xanthine derivatives including a styryl group at C8 is the most potent and selective.\textsuperscript{26} Recently Muller et al. reported that brominated and chlorinated styrylxanthine derivatives, (E)-8-(3-bromostyryl)-3,7-dimethyl-1-propargylxanthine (BS-DMPX) and (E)-8-(3-chlorostyryl)-3,7-dimethyl-1-propargylxanthine, had similar affinity for the adenosine A2A receptors as KF17837.\textsuperscript{27,28} We expected that the iodinated derivative, (E)-3,7-dimethyl-8-(3-iodostyryl)-1-propargylxanthine (IS-DMPX), may also have a high affinity for the A2A receptors.

In the present study, we prepared \(^{11}\text{C}\)-labeled IS-DMPX and BS-DMPX and evaluated their potential as \textit{in vivo} radioligands for mapping the adenosine A2A receptors in the CNS. BS-DMPX is potentially labeled with another positron-emitter such as \(^{82}\text{Br}\), and the \(^{123}\text{I}\)-labeled IS-DMPX can be applied to studies with single-photon emission computed tomography (SPECT). We measured the regional brain distribution of the tracers in mice by tissue dissection and \textit{ex vivo} autoradiography, and determined the receptor-specific binding \textit{in vivo} by a blocking study.

MATERIALS AND METHODS

Materials

An adenosine A2A receptor antagonist KF17837 and an adenosine A1 antagonist 8-dicyclopentylmethyl-1,3-dipropargylxanthine (KF15372) were prepared by Kyowa Hakko Kogyo Company.

Synthesis of IS-DMPX, BS-DMPX and their 7-demethyl derivatives

To a stirred suspension of 3-iodocinnamic acid (8.8 g) in methylene chloride (210 mL) were added thionyl chloride (2.8 mL) and pyridine (3 mL) at 0°C, and the reaction mixture was stirred for a further 3 hours at room temperature and concentrated. The residue was dissolved in \(N,N\)-dimethylformamide (160 mL), and 5,6-diamino-1-methyluracil (5 g) and pyridine (3.7 mL) was added to the mixture. The reaction mixture was stirred overnight and poured into ice-water (800 mL). Neutralization (pH 6) gave (E)-6-amino-1-methyl-5-(3-iodocinnamamoyl-amino)uracil (9.4 g) as a colorless powder. To the solution of the uracil obtained (9.0 g) in \(N,N\)-dimethylformamide (500 mL) were added CS\(_2\)CO\(_3\) (8.5 g) and propargyl bromide (5.4 mL, 80 w/w\% toluene solution), and the reaction mixture was stirred for 3 days at room temperature. The mixture was poured into ice-water (1 L), and the crude material obtained was collected. Recrystallization from ethanol gave (E)-6-amino-1-methyl-5-(3-iodocinnamamoylamin)-3-propargyluracil (2.6 g) as a colorless powder. The mixture of the obtained uracil (2.6 g), aqueous 4 N NaOH (160 mL), and ethanol (180 mL) was refluxed for 2 hours. The mixture was poured into ice-water (500 mL) and neutralized with conc. HCl (pH 6.2). Recrystallization of the crude material obtained from dioxane-water gave (E)-3-methyl-8-(3-iodostyryl)-1-propargylxanthine (0.6 g, 7-demethyl IS-DMPX) as a colorless powder: mp \(>270°C\); \(^1\text{H}\) NMR (270 MHz, CF\(_3\)CO\(_2\)D) \(\delta\) ppm: 11.35 (1H, s), 7.92 (1H, s), 7.82 (1H, d, J = 7.9 Hz), 7.71 (1H, d, J = 16.5 Hz), 7.56 (1H, d, J = 7.9 Hz), 7.26–7.19 (1H, m), 7.07 (1H, d, J = 16.5 Hz), 4.87 (2H, d, J = 2.6 Hz), 3.75 (3H, s), 2.26 (1H, t, J = 2.6 Hz); Elementary Analysis (C\(_7\)H\(_7\)N\(_2\)O\(_3\)); Caled for (%): C, 47.24; H, 3.03; N, 12.96, found (%): C, 47.57; H, 3.06; N, 12.99.

To a suspension of 7-demethyl IS-DMPX (455 mg) in \(N,N\)-dimethylformamide (20 mL) was added K\(_2\)CO\(_3\) (400 mg) and methyl iodide (0.1 mL) and the mixture was stirred for a hour at 50°C. The mixture was poured into ice-water (100 mL), and the crude material obtained was collected. Recrystallization from dioxane-water gave IS-DMPX (400 mg) as a pale yellow powder: mp 215–218°C (decomp); \(^1\text{H}\) NMR (270 MHz, CF\(_3\)CO\(_2\)D) \(\delta\) ppm: 7.97 (1H, s), 7.84 (1H, d, J = 9.6 Hz), 7.79 (1H, d, J = 16.5 Hz), 7.50 (1H, d, J = 7.6 Hz), 7.26–7.19 (1H, m), 6.93 (1H, d, J = 16.5 Hz), 4.84 (2H, d, J = 2.6 Hz), 4.26 (3H, s), 3.72 (3H, s), 2.24 (1H, t, J = 2.6 Hz); Elementary Analysis (C\(_7\)H\(_7\)N\(_2\)O\(_3\)); Caled for (%): C 48.45, H 3.39, N 12.56; Found (%): C 48.24, H 3.43, N 12.24.

BS-DMPX and 7-demethyl BS-DMPS were prepared as previously described.\textsuperscript{27}

Radiosynthesis of \([\textit{11}\text{C}]\text{IS-DMPX}\) and \([\textit{11}\text{C}]\text{BS-DMPX}\)

\([\textit{11}\text{C}]\text{IS-DMPX}\) and \([\textit{11}\text{C}]\text{BS-DMPX}\) were prepared by \textit{11}C-methylation of the corresponding 7-demethyl compounds with \([\textit{14}\text{C}]\)methyl iodide in the presence of Cs\(_2\)CO\(_3\) by the known method used for the synthesis of \([\textit{11}\text{C}]\)KF17837 and \([\textit{11}\text{C}]\)KF18446,\textsuperscript{19,23} but the radiochemical yields were very low (0.7–4.2%) based on \([\textit{11}\text{C}]\)methyl.
Fig. 1 Chemical structures of $[^{14}]$CJS-DMPX and $[^{14}]$CBS-DMPX. The affinity for adenosine A$_{2A}$ receptors was measured in the present study.

| Table 1 | Tissue distribution of radioactivity after intravenous injection of $[^{14}]$CJS-DMPX into mice |
|----------------|----------------------------------|----------------|----------------|----------------|
|                | Uptake (% ID/g)                  |                 |                 |                 |
| 5 min          | 15 min                           | 30 min          | 60 min          |
| Blood          | 0.40 ± 0.06                      | 0.29 ± 0.02     | 0.20 ± 0.03     | 0.22 ± 0.02     |
| Brain          | 1.88 ± 0.26                      | 1.77 ± 0.18     | 1.63 ± 0.28     | 1.22 ± 0.04     |
| Heart          | 15.19 ± 1.42                     | 10.98 ± 1.56    | 7.89 ± 0.58     | 5.19 ± 0.56     |
| Lung           | 2.73 ± 0.32                      | 1.88 ± 0.20     | 1.61 ± 0.24     | 1.29 ± 0.21     |
| Liver          | 5.65 ± 1.31                      | 4.32 ± 0.47     | 2.96 ± 0.37     | 3.14 ± 0.11     |
| Pancreas       | 3.33 ± 0.45                      | 2.18 ± 0.38     | 1.48 ± 0.30     | 0.94 ± 0.19     |
| Spleen         | 2.02 ± 0.20                      | 1.13 ± 0.29     | 0.94 ± 0.19     | 0.74 ± 0.16     |
| Kidney         | 11.48 ± 1.82                     | 10.55 ± 0.56    | 8.47 ± 0.79     | 5.34 ± 0.25     |
| Small intestine| 7.91 ± 0.74                      | 11.91 ± 0.84    | 8.11 ± 1.80     | 7.37 ± 2.04     |
| Muscle         | 3.09 ± 0.54                      | 2.59 ± 0.46     | 2.14 ± 0.07     | 2.10 ± 0.15     |

Mean ± s.d. (n = 4)

| Table 2 | Tissue distribution of radioactivity after intravenous injection of $[^{14}]$CBS-DMPX into mice |
|----------------|----------------------------------|----------------|----------------|----------------|
|                | Uptake (% ID/g)                  |                 |                 |                 |
| 5 min          | 15 min                           | 30 min          | 60 min          |
| Blood          | 0.42 ± 0.08                      | 0.35 ± 0.06     | 0.32 ± 0.03     | 0.23 ± 0.03     |
| Brain          | 2.46 ± 0.24                      | 2.35 ± 0.27     | 2.25 ± 0.21     | 1.79 ± 0.25     |
| Heart          | 15.45 ± 3.23                     | 11.20 ± 1.43    | 9.00 ± 1.30     | 5.63 ± 2.08     |
| Lung           | 2.70 ± 0.45                      | 2.07 ± 0.39     | 1.92 ± 0.51     | 1.46 ± 0.37     |
| Liver          | 5.44 ± 0.35                      | 5.91 ± 1.04     | 4.72 ± 0.60     | 3.24 ± 0.70     |
| Pancreas       | 3.13 ± 0.22                      | 2.68 ± 0.71     | 1.64 ± 0.40     | 1.00 ± 0.39     |
| Spleen         | 1.24 ± 0.24                      | 1.38 ± 0.24     | 1.07 ± 0.11     | 0.60 ± 0.03     |
| Kidney         | 10.33 ± 0.90                     | 11.31 ± 2.36    | 8.20 ± 0.97     | 5.34 ± 1.56     |
| Small intestine| 5.39 ± 1.16                      | 5.81 ± 1.18     | 5.50 ± 1.68     | 7.51 ± 1.65     |
| Muscle         | 3.40 ± 0.49                      | 2.97 ± 0.68     | 2.81 ± 0.55     | 2.05 ± 0.60     |

Mean ± s.d. (n = 4)

iodide). The specific radioactivity was 24–56 GBq/μmol.

Male ddY mice (8 weeks old) were obtained from Tokyo Laboratory Animals Company (Tokyo, Japan). The animal studies were approved by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Gerontology.

In vitro affinity for the adenosine A$_{2A}$ and A$_{1}$ receptors
The in vitro affinity of IS-DMPX and BS-DMPX for the adenosine A$_{2A}$ and A$_{1}$ receptors was determined by using rat striatal membrane and [carboxyethyl][H(N)]-2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamido adenosine hydrochloride as a radioligand and the rat forebrain membrane and N$^{6}$-[H]cyclohexyladenosine, respectively, as described. All assays were performed in the dark to prevent photoisomerization.

Biodistribution in mice
Each $^{[14]}$C-labeled tracer (1.0 MBq/25–49 pmol) was intravenously injected into mice. They were killed by cervical dislocation at 5, 15, 30 and 60 min post injection. The blood was collected by heart puncture, and the tissues were harvested and the brain was divided into the striatum, cerebellum, cortex and the rest. The $^{[14]}$C in the samples was counted with an auto-gamma counter and the tissues were weighed. The tissue uptake of $^{[14]}$C was expressed as
the percent injected dose per gram tissue (%ID/g).

In another group of mice, the tracer was co-injected together with one of three adenosine antagonists: carrier IS-DPMX or BS-DMPX, an adenosine A2A antagonist KF17837\textsuperscript{26} and an A1 antagonist KF15372,\textsuperscript{23} and the radioactivity levels in the regional brain and blood were measured at 15 min after injection. The amount of the co-injected compounds was 50 nmol/animal.

Ex vivo autoradiographic study
Mice were killed 30 min after intravenous injection of \([^{14}C]IS\)-DMPX (46 MBq/1.4 pmol) or \([^{14}C]BS\)-DMPX (57 MBq/1.3 pmol). The brain was rapidly dissected, frozen and sagittally cut into 20 µm-thick sections with a cryotome (Bright Instrument Co., Ltd., Huntingdon, UK). The brain sections were dried on a hot plate at 60°C and were exposed to a storage phosphor screen (PhosphorImager SI system, Molecular Dynamics, Sunnyvale CA, USA) until complete decay. The regional brain distribution was visualized.

RESULTS

In vitro affinity for the adenosine A\textsubscript{2A} receptors
The Ki values of IS-DMPX were 8.9 ± 0.87 nM for the adenosine A\textsubscript{2A} receptors and >10000 nM for the adenosine A\textsubscript{1} receptors. The Ki values of BS-DMPX were 7.7 ± 0.37 nM for the adenosine A\textsubscript{2A} receptors and 2300 ± 120 nM for the adenosine A\textsubscript{1} receptors. The two compounds had a similar affinity for A\textsubscript{2A} receptors, but IS-DMPX was more selective for the A\textsubscript{2A} receptors than BS-DMPX: A\textsubscript{2A}/A\textsubscript{1} ratios were >1100 and 300 for IS-DMPX and BS-DMPX, respectively.

Tissue distribution of \([^{14}C]IS\)-DMPX and \([^{14}C]BS\)-DMPX in mice
\([^{14}C]IS\)-DMPX and \([^{14}C]BS\)-DMPX showed a similar tissue distribution (Tables 1 and 2). Blood clearance of the radioactivity was very rapid. At 5 min after the injection, the highest uptake of the two tracers was found in the heart, followed by the kidney, liver or small intestine. In all tissues the radioactivity levels were decreased after 15 min except for the uptake of \([^{14}C]BS\)-DMPX by the small intestine. The brain uptake was low but relatively retained for 60 min among the tissues investigated.

Figure 2 shows the regional brain uptake and the uptake ratio of the striatum to reference tissues. The uptake of the two tracers was slightly higher in the striatum than in the cortex and cerebellum. The uptake ratios of the striatum to reference regions were slightly increased but low. The striatum-to-cortex and striatum-to-cerebellum ratios were 1.6 ± 0.2 and 1.2 ± 0.2, respectively, for \([^{14}C]IS\)-DMPX and 1.7 ± 0.3 and 1.2 ± 0.2, respectively, for \([^{14}C]BS\)-DMPX at 60 min postinjection.

As shown in Figure 3, the uptake of \([^{14}C]BS\)-DMPX by these three regions was significantly reduced by co-
injection of 50 nmol of carrier or an adenosine $A_{2A}$ antagonist KF17837 (43–68% of the control). It is noted that the uptake by the three regions was significantly increased by an $A_1$ antagonist KF15372. The blocking effect of the uptake of $[^{11}C]$JS-DMPX was smaller (71–94% of the control), and only the cortex and cerebellum with KF17837 showed a statistically significant reduction.

Ex vivo autoradiographic study
Figure 4 shows the ex vivo autoradiographic image of a sagittal brain section of the mouse at 30 min after the tracer injection. A slightly high uptake of $[^{11}C]$JS-DMPX and $[^{11}C]$BS-DMPX was found in the striatum, but a high uptake was also observed in other brain regions as shown on tissue dissection.

DISCUSSION
From the first proposal of KF17837 by Shimada et al., several xanthine derivatives have been widely used as selective antagonists for adenosine $A_{2A}$ receptors. Recently we found that $[^{11}C]$KF18446 is a promising PET ligand for mapping adenosine $A_{2A}$ receptors. In the present study, we prepared $[^{11}C]$-labeled new xanthine derivatives containing iodine or bromine and evaluated their in vivo characteristics for the purpose of developing radioligands for studying adenosine $A_{2A}$ receptors with PET and SPECT.

In the present membrane binding studies, the two ligands have a similar affinity for $A_{2A}$ receptor: 8.9 ± 0.87 nM for IS-DMPX and 7.7 ± 0.37 nM for BS-DMPX. The affinity of BS-DMPX was comparable to the previously reported value (Ki, 8.2 nM for $A_{2A}$ receptors and 1200 nM for the $A_1$ receptors). We found that IS-DMPX showed better $A_{2A}/A_1$ selectivity than BS-DMPX and other $A_{2A}$ ligands: 300 for IS-DMPX versus 1100 for BS-DMPX. As compared with $[^{11}C]$KF18446, which is a previously proposed PET ligand, the two ligands had a slightly lower affinity for $A_{2A}$ receptors than KF18446, and IS-DMPX showed more selectivity than KF18446: the Ki value of KF18446 for the $A_{2A}$ receptors was 5.9 nM, and $A_{2A}/A_1$ selectivity was 270.

In mice both tracers were taken up slightly higher by the striatum than by the cortex and cerebellum, but the uptake by all three regions was reduced by co-injection of carrier or an adenosine $A_{2A}$ receptor antagonist KF17837, suggesting specific uptake by the cortex and cerebellum as well as by the striatum. The uptake was unexpectedly...
increased by co-injection of an A1 antagonist possibly due to a pharmacological effect. The blocking effects were small: 6–29% for [14C]5-DMX and 32–57% for [14C]-5-DMX at 15 min postinjection, suggesting that [14C]5-DMX shows a more receptor-specific uptake than [14C]5-DMX. It is well established that the adenosine A2A receptors are highly enriched in the striatum, although recent studies also demonstrated the presence of atypical A2A receptors in the hippocampus and cortex. The uptake ratios of striatum to cortex and striatum to cerebellum for [14C]5-DMX or [14C]5-DMX were much smaller than those for [14C]5-DMX previously proposed.23 These findings suggest that the selectivity of the tracers is not suitable for in vivo ligands for studying adenosine A2A receptors in the CNS. On the other hand, peripherally both tracers were highly taken up by the heart (Tables 1 and 2), suggesting that they may be used in myocardial imaging as [14C]5-DMX.21

In conclusion, we prepared iodinated and brominated xanthine derivatives, IS-5-DMX and BS-5-DMX, and their [14C]-labeled analogs. In vitro binding assay showed that two ligands have a high and selective affinity for adenosine A2A receptors. Nevertheless, the two [14C]-labeled tracers were not suitable ligands for studying A2A receptors of the CNS in vivo, because of the low selectivity evaluated by the uptake ratios of the striatum to reference regions and because of a high nonspecific binding.

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