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Potential of an adenosine A_{2A} receptor antagonist [^{11}C]TMSX for myocardial imaging by positron emission tomography: a first human study

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In previous *in vivo* studies with mice, rats, cats and monkeys, we have demonstrated that [7-methyl-^{11}C]-(E)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine ([^{11}C]TMSX) is a potential radioligand for mapping adenosine A_{2A} receptors of the brain by positron emission tomography (PET). In the present study, we studied the potential of [^{11}C]TMSX for myocardial imaging. Uptake of radioactivity by the heart was high and gradually decreased after an intravenous injection of [^{11}C]TMSX into mice. In metabolite analysis, 54% and 76% of the radioactivity in plasma and heart, respectively, were present as the unchanged form of [^{11}C]TMSX 60 min postinjection. The myocardial uptake was reduced by carrier-loading and by co-injection of an adenosine A_{2A} antagonist CSC, but not by co-injection of an adenosine A_1 antagonist DPCPX. Pretreatment with a high dose of a non-selective antagonist theophylline also reduced the myocardial uptake of [^{11}C]TMSX. These findings demonstrate the specific binding of [^{11}C]TMSX to adenosine A_{2A} receptors in the heart. Finally we successfully performed the myocardial imaging by PET with [^{11}C]TMSX in a normal volunteer. A graphical analysis by Logan plot supported the receptor-mediated uptake of [^{11}C]TMSX. Peripherally [^{11}C]TMSX was very stable in human: >90% of the radioactivity in plasma was detected as the unchanged form in a 60-min study. We concluded that [^{11}C]TMSX PET has the potential for myocardial imaging.

Key words: adenosine A_{2A} receptor, [^{11}C]TMSX, heart, human, positron emission tomography

INTRODUCTION

ADENOSINE is an endogenous modulator of several physiological functions in the central nervous system (CNS) as well as in peripheral organs.[1–6] The effects are mediated by at least four subtypes: A_1, A_{2A}, A_{2B} and A_3 receptors. In the CNS the adenosine A_{2A} receptors are highly enriched in the striatum, nucleus accumbens and olfactory tubercle,[7,8] in which adenosine A_{2A} and dopamine D_2 receptors are co-expressed on GABAergic-enkephaline neurons.[9,10] Therefore, the adenosine A_{2A} receptor can be used as an alternative target maker as the dopamine D_2 receptor for characterizing the degeneration of pre-synaptic nigrostriatal and post-synaptic striatopallidal neurons of the stratum in the patients with neurological disorders by positron emission tomography (PET).[11] Based on the this background, we have developed several positron-emitting ligands for mapping the CNS adenosine A_{2A} receptors,[12–19] and proposed [7-methyl-^{11}C]-(*E*)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine ([^{11}C]TMSX, Fig. 1, abbreviated as [^{11}C]KF18446 previously) as a candidate in PET studies.[11,15,16,19]

In previous studies on the tissue distribution of [^{11}C]TMSX in mice, we found that the uptake level of [^{11}C]TMSX in the heart was high and with the level gradually decreasing.[15] This finding suggests the adenosine A_{2A} receptor-mediated uptake of [^{11}C]TMSX in the heart. We also reported that an analogue of [^{11}C]TMSX, [7-methyl-^{11}C]-(*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine ([^{11}C]KF17837), had the potential for imaging adenosine A_{2A} receptors in the heart.[20] Therefore, in the present study we investigated the specific

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binding of [^{11}C]TMSX to the adenosine A_{2A} receptors in the heart to clarify whether the tracer has potential as a new diagnostic probe by PET. Furthermore, because we previously evaluated the suitability of [^{11}C]TMSX in a clinical context,[21] we also performed the first trial of imaging of the human heart by [^{11}C]TMSX and PET.

MATERIALS AND METHODS

8-Chlorostyryl-1,3,7-trimethylxanthine (CSC), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and theophylline were purchased from Research Biochemical, Inc. (Natick, MA, USA). (*E*)-1,3-dimethyl-8-(3,4,5-trimethoxystyryl)xanthine and TMSX were synthesized in our laboratory as previously described.[22] [^{11}C]TMSX was prepared by methylation of (*E*)-1,3-dimethyl-8-(3,4,5-trimethoxystyryl)xanthine with [^{11}C]methyl iodide using a previously described method.[15] Male ddY mice (8-week-old) were obtained from Tokyo Laboratory Animals Co., Ltd. (Tokyo, Japan). The animal study was approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology. The clinical study was also approved by the institutional Ethical Committee, and a written informed consent was obtained from the subject.

Myocardial uptake and metabolism of [^{11}C]TMSX in mice

[^{11}C]TMSX (10 MBq/140 pmol) was intravenously injected into mice (8 weeks old). They were killed by cervical dislocation 5, 15, 30 and 60 min after the injection (n = 4). Blood was collected by heart puncture using a heparinized syringe, and the heart, lung and muscle were harvested. The samples were measured for the ^{11}C-radioactivity with an auto-gamma counter and weighed. The tissue uptake of radioactivity was expressed as a percentage of the injected dose per gram of tissue (%ID/g).

Metabolite analysis were was carried out under dim light to prevent photoisomerization of [^{11}C]TMSX as described previously.[15] Briefly the plasma and heart were homogenized in acetonitrile containing trichloroacetic acid, and the acid-soluble fraction was analyzed by high-performance liquid chromatography (n = 3).

Effects of adenosine receptor antagonists on the myocardial uptake of [^{11}C]TMSX in mice

In the first group of mice, the effect of carrier doses on the myocardial uptake of [^{11}C]TMSX was examined. [^{11}C]TMSX (2.0 MBq/68 pmol, 1.9 nmol/0.75 μg/kg) was co-injected with different amounts of carrier TMSX into mice (n = 4–5). The co-injected doses of TMSX were 0.0033, 0.011, 0.033, 0.11, 0.33 and 1.1 mg/kg (8.6, 29, 86, 290, 860 or 2900 nmol/kg). In the second group of mice, [^{11}C]TMSX (2.0 MBq/45 pmol, 1.3 nmol/0.50 μg/kg) was co-injected together with an adenosine A_{2A} antagonist CSC or an adenosine A_1 antagonist DPCPX (n = 4–5). The co-injected dose of each compound was 100 nmol/animal (2900 nmol/kg). In the third group of mice, [^{11}C]TMSX (2.0 MBq/25 pmol, 0.71 nmol/0.28 μg/kg) was intravenously injected 15 min after the intraperitoneal injection of theophylline, a non-selective antagonist, at the doses of 1, 10 and 100 mg/kg (3.0, 30 or 300 μmol/kg) (n = 4–5). The K_i values of the compound used for adenosine A_1 and A_{2A} receptors were 1600 nM and 5.9 nM for TMSX;[15,16] 28000 nM and 54 nM for CSC;[23] and 23000 nM and 16000 nM for theophylline;[22] and 6.5 nM and 590 nM for DPCPX.[22] The mice were killed 15 min after injection, the blood and heart were obtained, and the level of radioactivity was measured as the %ID/g.

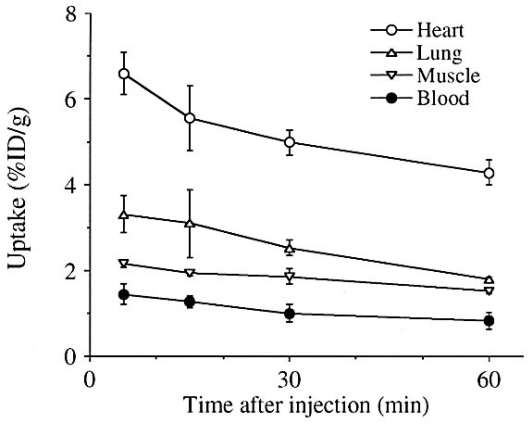

Fig. 1 Levels of radioactivity in the heart, lung, muscle and plasma after intravenous injection of [^{11}C]TMSX into mice. Mean ± sd (n = 4). Injected dose of [^{11}C]TMSX was 10 MBq/140 pmol.

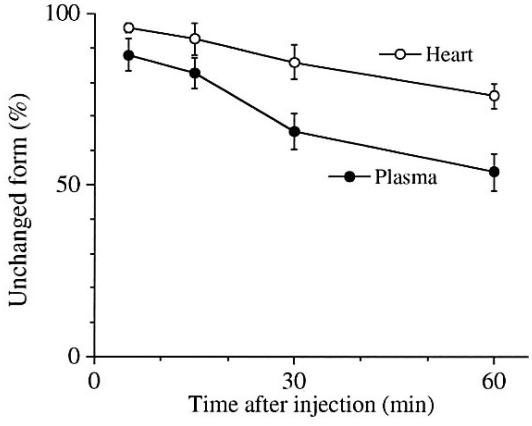

Fig. 2 Percentages of the unchanged form after intravenous injection of [^{11}C]TMSX into mice. Mean ± sd (n = 3).

Myocardial imaging by [^{11}C]TMSX PET in a human

A 26-year-old male normal volunteer underwent PET scans with [^{11}C]TMSX. PET measurement was performed with SET-2400W (Shimadzu Co., Kyoto, Japan). After transmission scan with a rotating [^{68}Ga]/[^{68}Ge] line source

to correct attenuation, [^{11}C]TMSX (590 MBq/15 nmol) was injected intravenously into the subject, and PET scan was performed for 60 min in a dynamic mode (10 sec × 6 frames, 30 sec × 3 frames, 60 sec × 5 frames, 150 sec × 5 frames, 300 sec × 8 frames). The tomographic images were reconstructed using a filtered backprojection method, cutoff frequency 144 cycle/cm and order 2. The data were collected in a 128 × 128 × 33 matrix. The voxel size was 2 × 2 × 6.25 mm. Arterial blood was taken at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 145, 160, 175, and 180 sec, and 5, 7, 10, 15, 20, 30, 40, 50, and 60 min, and the plasma radioactivity was measured. The unchanged form of [^{11}C]TMSX in the plasma sampled at 3, 10, 20, 30, 40, and 60 min was analyzed by high-performance liquid chromatography.[15]

Regions of interest (ROIs) were placed on the left ventricular anterior wall, interventricular septum, left ventricular lateral wall, lung and muscle. Time-activity curves (TACs) in these ROIs and in plasma were calculated as Bq/m*l* or the standardized uptake value (SUV, g body weight × Bq/m*l* tissue/total injected dose). Using the TACs in the left ventricular lateral wall, left ventricular anterior wall and interventricular septum, and the metabolite-corrected TAC in plasma, the distribution volume for [^{11}C]TMSX in the three regions of the heart was evaluated using a graphical analysis by Logan et al.[24]

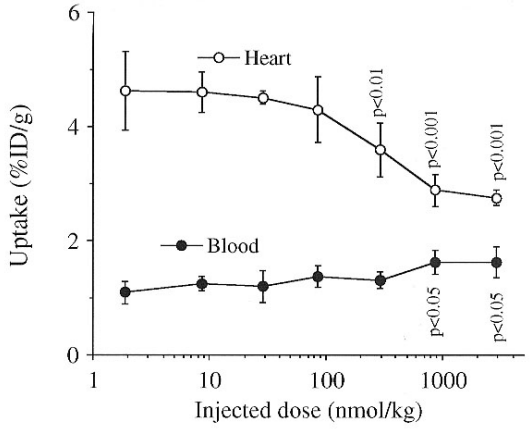

Fig. 3 Effects of carrier-loading on the myocardial uptake of radioactivity 15 min after intravenous injection of [^{11}C]TMSX into mice. Injected dose of [^{11}C]TMSX was 2.0 MBq/68 pmol (1.9 nmol/0.75 μg/kg) (control level). Co-injected doses of TMSX were 0.0033, 0.011, 0.033, 0.11, 0.33 and 1.1 mg/kg (8.6, 29, 86, 290, 860 or 2900 nmol/kg). Mean ± sd (n = 4–5). Student's t-test was performed compared to the control level.

RESULTS

Figure 1 shows time-activity curves of the heart, lung, muscle and blood in mice. The level of radioactivity in the heart was higher than those in three other tissues and

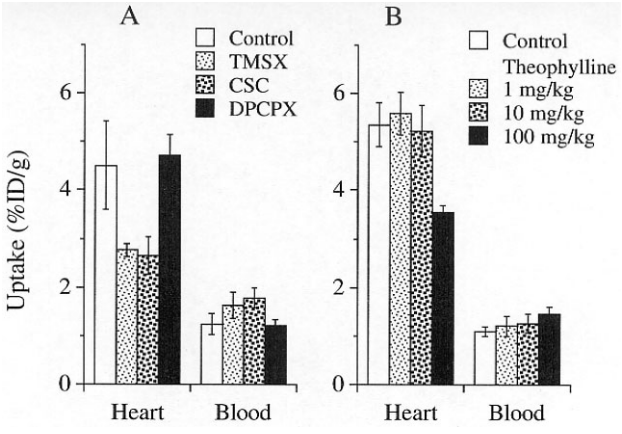

Fig. 4 Effects of co-injection of adenosine A_{2A}-selective CSC or adenosine A_1-selective DPCPX (A) and of pretreatment with non-selective theophylline (B) on the myocardial uptake 15 min after intravenous injection of [^{11}C]TMSX into mice. A: Injected dose of [^{11}C]TMSX was 2.0 MBq/45 pmol (1.3 nmol/0.50 μg/kg), and co-injected dose of antagonists was 100 nmol/animal (2900 nmol/kg). B: Injected dose of [^{11}C]TMSX was 2.0 MBq/25 pmol (0.71 nmol/0.28 μg/kg), and pre-treated doses of theophylline 15 min before the tracer injection were 1, 10 and 100 mg/kg (3, 30 and 300 μmol/kg). Mean ± sd (n = 4–5). Student's t-test was performed compared to the control.

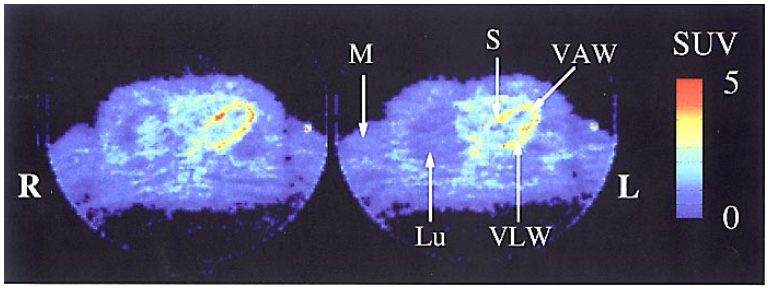

Fig. 5 PET images of radioactivity in the chest region of a human after intravenous injection of [^{11}C]TMSX. The [^{11}C]TMSX PET images were acquired for 25 min starting at 10 min after injection. The radioactivity level was expressed as the standardized uptake value (SUV, g body weight × Bq/m*l* tissue/total injected dose). L, left side; R, right side; VLW, left ventricular lateral wall; VAW, left ventricular anterior wall; S, interventricular septum; Lu, lung; and M, brachialis muscle.

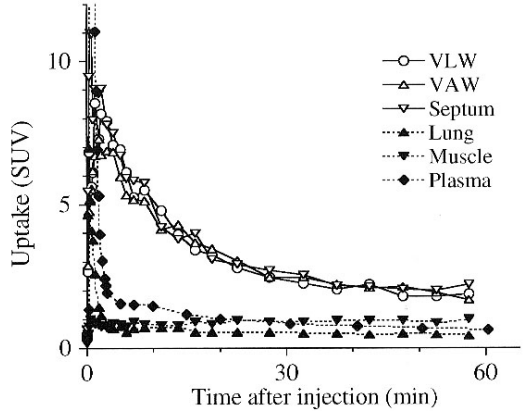

Fig. 6 Time-activity curves of [^{11}C]TMSX in the heart, lung, muscle and plasma in a human. The time-activity curves in the left ventricular lateral wall and brachialis muscle are presented as those in the heart and muscle, respectively.

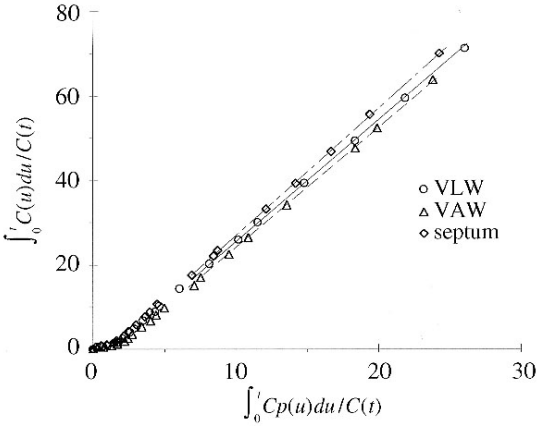

Fig. 7 Graphical analysis of [^{11}C]TMSX in the human heart using Logan plot.[24] Slopes of fits represent the distribution volumes: $y = 2.8481x - 2.8023$ ($R^2 = 1.0000$) for left ventricular lateral wall (VLW); $y = 2.8702x - 4.6506$ ($R^2 = 0.9998$) for left ventricular anterior wall; and $y = 3.0254x - 3.1807$ ($R^2 = 0.9998$) for ventricular septum. *C*, activity in tissue; and *Cp*, activity in plasma; *t*, elapsed time.

decreased gradually. Metabolite analysis showed that the unchanged form gradually decreased in the heart and plasma, but that 76% of the radioactivity in the heart remained as [^{11}C]TMSX 60 min postinjection (Fig. 2).

The specific binding of [^{11}C]TMSX to adenosine A_{2A} receptors in the heart was investigated by the uptake level 15 min after intravenous injection of [^{11}C]TMSX. The uptake of the radioactivity decreased dose-dependently (Fig. 3). The uptake was also reduced by co-injection of an adenosine A_{2A} receptor antagonist CSC, but not by co-injection of an adenosine A_1 receptor antagonist DPCPX (Fig. 4A). When a large amount of theophylline with nonselective and low affinity for adenosine receptors was pre-treated, the uptake was also significantly decreased (Fig. 4B).

Figure 5 shows PET images with [^{11}C]TMSX in a human. The heart was clearly visualized. The levels of radioactivity in three regions of the heart increased for the first 2.5 min after the injection, and then decreased gradually (Fig. 6). In marked contrast to the heart the radioactivity was rapidly washed out much faster in the lung. The level in the muscle was low, and remained constant. Percentages of the unchanged form in plasma were 97.3%, 96.7%, 95.2%, 94.4%, 93.1% and 94.6% at 3, 10, 20, 30, 40 and 60 min, respectively. The distribution volumes evaluated by the graphical analysis (Fig. 7) were 2.8, 2.9 and 3.0 in the left ventricular lateral wall, left ventricular anterior wall and interventricular septum, respectively.

DISCUSSION

The present study clearly demonstrated that the uptake of [^{11}C]TMSX in the heart was mediated by adenosine A_{2A} receptors. We carried out blocking studies to evaluate the receptor-specific uptake of [^{11}C]TMSX 15 min postinjection, because the uptake in the heart and the unchanged form gradually decreased with time. A dose-dependent decrease in the uptake of radioactivity demonstrates the presence of saturable binding of [^{11}C]TMSX. Blockade of the uptake of [^{11}C]TMSX with adenosine A_{2A}-selective CSC and non-selective theophylline, but not with A_1-selective DPCPX, reflects the specific binding of [^{11}C]TMSX to adenosine A_{2A} receptors in the heart. At least 40% of the uptake was estimated as the A_{2A} receptor-mediated uptake by the blockade with carrier TMSX at 1.1 mg/kg (2900 nmol/kg) and CSC at 0.94 mg/kg (2900 nmol/kg) 15 min postinjection. A lower blockade (34%) with theophylline at 100 mg/kg was due to the weaker affinity for the adenosine A_{2A} receptors.

PET imaging of the human heart was successfully performed in a volunteer (Fig. 6). Time-activity curves in the three regions of the heart and the graphical analysis by Logan plot clearly demonstrated the retention of [^{11}C]TMSX that suggests the receptor-mediated uptake. It is pointed out that peripherally [^{11}C]TMSX was much more stable in the human subject than in mice. In mice the percentages of the unchanged form gradually decreased to 54% and 76% in plasma and heart, respectively (Fig. 2), while >90% of the radioactivity in human plasma was detected as the unchanged form in a 60-min study. These findings suggest that most signal in the human heart was due to the unchanged [^{11}C]TMSX. As for the distribution volumes for [^{11}C]TMSX, there was no regional difference in the three regions of the heart.

In the cardiovascular system, traditionally, the adenosine A_1 receptors have been thought to be the only subtype expressed in ventricular cardiomyocytes. Activation of the adenosine A_1 receptors has been reported to elicit bradycardia, depression of myocardial contractility and reduction of impulse conduction velocity. On the other hand, the adenosine A_2 receptors are present on the

endothelium and on the vascular smooth muscle cells, mediating the endothelium-dependent and -independent vasodilation, respectively. Although conflicting data exist regarding the presence and function of adenosine A_2 receptors on the cardiac myocytes, there is increasing evidence, however, that other adenosine receptor subtypes also exist in ventricular myocytes and that they may have important physiological functions.[2] Xu et al. have clearly shown that A_{2A} receptors are expressed and are functionally coupled to the stimulation of cAMP accumulation and cardiac contractility in adult rat ventricular myocytes.[25] Norton et al. proposed that A_{2A} receptors are expressed in rat ventricular cardiomyocytes and that they serve to counteract the antiadrenergic effects of adenosine A_1 receptors.[26] Kilpatrick et al. supported the expression of the adenosine A_{2A} receptors in rat ventricular myocytes.[27] Further investigations as to whether the uptake of [^{11}C]TMSX reflects the binding sites in the endothelium and/or ventricular myocytes are necessary, but diagnosis of ischemia and other myocardial diseases by the [^{11}C]TMSX PET is of great interest because of the cardiovascular function of adenosine receptors.

Clinically theophylline is used as an antasthmatic. It has a much lower affinity for the adenosine A_{2A} receptors than TMSX. Pretreatment with theophylline at 100 mg/kg significantly reduced the uptake of [^{11}C]TMSX by the heart. Although the clinical dosage of theophylline is estimated to be in the range of 1–10 mg/kg, it is noted that [^{11}C]TMSX PET should be especially carefully performed in patients receiving theophylline.

In conclusion, the present study demonstrated that [^{11}C]TMSX binds to adenosine A_{2A} receptors in the heart, and [^{11}C]TMSX PET has the potential for myocardial imaging.

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