

## Preclinical evaluation of [ $^{11}\text{C}$ ]SA4503: radiation dosimetry, *in vivo* selectivity and PET imaging of sigma $_1$ receptors in the cat brain

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Our previous *in vivo* study with rats has demonstrated that  $^{11}\text{C}$ -labeled 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine ([ $^{11}\text{C}$ ]SA4503) is a potential radioligand for mapping CNS sigma $_1$  receptors by positron emission tomography (PET). In the present study, we further characterized this ligand. The radiation absorbed-dose of [ $^{11}\text{C}$ ]SA4503 in humans estimated with the tissue distribution in mice, was higher in the liver, kidney and pancreas than in other organs studied, but was low enough for clinical use. The brain uptake of [ $^{11}\text{C}$ ]SA4503 in mice was reduced to approximately 60–70% by co-injection of carrier SA4503 and haloperidol, but not by co-injection of any of six ligands for sigma $_2$  or other receptors, for which SA4503 showed *in vitro* >100 times weaker affinity than for sigma $_1$  receptor. In the cat brain, the uptake in the cortex was higher than that in the cerebellum. The radioactivity in the cortex and cerebellum accumulated for the first 10 min and then gradually decreased until 81.5 min in the baseline measurement, but rapidly decreased in the carrier-loading condition. The receptor-mediated uptake was estimated to be approximately 60–65% of the total radioactivity in the cortex and cerebellum at 76 min after tracer injection. We have concluded that [ $^{11}\text{C}$ ]SA4503 has the potential for mapping sigma $_1$  receptor by PET.

**Key words:** sigma $_1$  receptor, [ $^{11}\text{C}$ ]SA4503, central nervous system, positron emission tomography

### INTRODUCTION

THE SIGMA $_1$  RECEPTOR is considered to be involved in some diseases of the central nervous system (CNS), such as schizophrenia,<sup>1,27</sup> depression,<sup>18</sup> dementia<sup>1,18,23</sup> and ischemia.<sup>23</sup> Furthermore, the sigma receptors have been found in the endocrine, immune and other peripheral organ systems and peripheral nervous systems,<sup>7,22,31,36,40</sup> and are also expressed in a variety of human tumors<sup>2,37</sup> such as neuroblastoma, glioma and melanoma.

Several radioligands have been synthesized and evaluated for imaging the sigma receptors by positron emission tomography (PET)<sup>3–5,9,16,17,26,33,34,38</sup> and by single photon

emission tomography.<sup>10–14,25,39</sup> In a previous study, we have found  $^{11}\text{C}$ -labeled [4- $O$ - $^{11}\text{C}$ -methyl]-1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride ([ $^{11}\text{C}$ ]SA4503, shown in Fig. 1) to be a promising PET ligand for mapping sigma $_1$  receptors.<sup>15</sup> In the *in vivo* study with rats, the brain uptake of [ $^{11}\text{C}$ ]SA4503 was high, and approximately 75–85% of the total radioactivity in the brain reflected the receptor-specific uptake.<sup>15</sup> No  $^{11}\text{C}$ -labeled metabolite was detected in the rat brain tissue, and metabolic alteration seemed to be slow: approximately 80% of the radioactivity was found as an unchanged form in plasma 30 min postinjection.<sup>15</sup> The regional brain distribution of [ $^{11}\text{C}$ ]SA4503 was clearly demonstrated by *ex vivo* ARG. In previous *in vitro* studies, SA4503 had a selective affinity for sigma $_1$  receptors (IC $_{50}$ , 17.4 nM; selectivity sigma $_1$ /sigma $_2$ , 103).<sup>6,19,20</sup> The SA4503 has weak affinity for  $\alpha_1$ -adrenargic, dopamine D $_2$ , serotonin (5-HT) $_{1A}$ , 5-HT $_2$ , histamine H $_1$ , muscarinic M $_1$ , and muscarinic M $_2$  receptors.<sup>19,20</sup> These affinities

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were over 100 times weaker than that for the sigma<sub>1</sub> receptor. The affinity may be low for the *in vivo* binding of [<sup>11</sup>C]SA4503 to the receptors. Moreover, SA4503 had no affinity for 29 other receptors (such as *N*-methyl-D-aspartate, phencyclidine and opiate), ion channels or second messenger systems.<sup>19,20</sup>

In the present study, we estimated the radiation dosimetry of [<sup>11</sup>C]SA4503 for humans from mice data and further characterized the *in vivo* selectivity of the compound as a sigma<sub>1</sub> receptor ligand. We evaluated whether [<sup>11</sup>C]SA4503 binds *in vivo* to lower affinity sites such as α<sub>1</sub>-adrenargic, dopamine D<sub>2</sub>, 5-HT<sub>2</sub>/5-HT<sub>1C</sub> histamine H<sub>1</sub> and cholinergic receptors. The study was performed in mice, because slight species difference in the density of sigma receptors was reported,<sup>30</sup> and because the receptor-specific binding of [<sup>11</sup>C]SA6298, an analog of [<sup>11</sup>C]SA4503, was slightly different in rats and mice.<sup>16</sup> We also performed imaging of sigma receptors in the cat brain by PET with [<sup>11</sup>C]SA4503.

## MATERIALS AND METHODS

1-(4-Hydroxy-3-methoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (4-*O*-demethyl SA4503) was synthesized in Tokyo Metropolitan Institute of Gerontology as previously described.<sup>6</sup> 1-(3,4-Dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503) was prepared by Santen Pharmaceutical Co. (Osaka, Japan). Raclopride tartrate was supplied by Astra Arcus AB (Södertälje, Sweden). Benoxathian hydrochloride, 1,3-di(2-tolyl)guanidine (DTG), haloperidol, pyrilamine malate and ritanserin were purchased from Research Biochemicals International (Natick, MA, USA). Atropine sulfate was obtained from Wako Chemical Industry Ltd. (Tokyo, Japan).

Male ddY mice were obtained from Tokyo Laboratory Animals Co., Ltd. (Tokyo, Japan). An adult male cat was supplied by IEFA CREDO (IOC cats, Lyon, France). The animal studies were approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology.

### Radiosynthesis

[<sup>11</sup>C]SA4503 (Fig. 1) was prepared by methylation of 4-*O*-demethyl SA4503 with [<sup>11</sup>C]CH<sub>3</sub>I in a solution of *N,N*-dimethylformamide containing NaH, followed by high performance liquid chromatography (HPLC) purification, according to the previously described method.<sup>15</sup> The specific activity was 24–76 TBq/mmol.

### Tissue distribution study

[<sup>11</sup>C]SA4503 (1.1–2.0 MBq/16–34 pmol) was intravenously injected into mice (8 weeks old). They were killed by cervical dislocation at 1, 5, 15, 30, 60 and 90 min after injection (n = 4). The blood was collected by heart puncture, and the tissues were harvested and weighed.

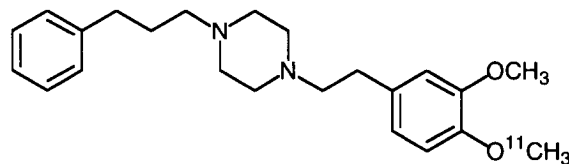


Fig. 1 Chemical structure of [<sup>11</sup>C]SA4503.

The <sup>11</sup>C in the samples was measured with an auto-gamma counter. The tissue uptake of <sup>11</sup>C was expressed as the percent injected dose per organ (%ID/organ), the percent injected dose per gram tissue (%ID/g) or the standardized uptake value (SUV, [tissue activity/gram tissue]/injected activity/gram body weight). Based on the mice data, radiation dosimetry for human adults was estimated by the MIRD method as described previously.<sup>8,24</sup>

### Blocking study

The effect of carrier doses on the brain uptake of [<sup>11</sup>C]SA4503 was examined. [<sup>11</sup>C]SA4503 was co-injected with different amounts of carrier SA4503 into mice. The co-injected doses of SA4503 were 7, 70, 700, 2000 or 5000 nmol/kg. The mice were killed at 30 min after injection, and the brain uptake was measured.

In another group of mice, [<sup>11</sup>C]SA4503 was co-injected with one of the following receptor ligands: haloperidol for sigma<sub>1</sub> receptor, DTG for sigma<sub>1</sub>/sigma<sub>2</sub> receptor, benoxathian for α<sub>1</sub> adrenoreceptor, raclopride for dopamine D<sub>2</sub> receptor, ritanserin for serotonin 5-HT<sub>2</sub>/5-HT<sub>1C</sub> receptor, pyrilamine for histamine H<sub>1</sub> receptor and atropine for cholinergic receptors. The co-injected dose of the ligands was 2000 nmol/kg except for DTG (1600 nmol/kg). The brain uptake of [<sup>11</sup>C]SA4503 was measured at 30 min postinjection.

### PET study on a cat

A cat weighing 4.4 kg was anesthetized with atropine (0.1 mL/kg) and thiopental sodium (15 mg/kg), and was placed in the prone position on a holder. Catheters were inserted into the femoral vein and femoral artery. [<sup>11</sup>C]SA4503 (200 MBq/57 nmol) was intravenously injected through the catheter, and an 85 min PET scan was done (one 30 sec frame, one 1 min frame, two 2 min frames, three 4 min frames, three 6 min frames, one 10 min frame and three 12 min frames). After the radioactivity decayed out, the tracer (190 MBq/19 nmol) was injected together with cold SA4503 (1.0 mg/kg), and the 85-min PET scan was done. The PET camera was a model SHR 2000 (Hamamatsu Photonics, Hamamatsu, Japan). The camera consists of four ring detectors and acquires seven slices at a center-to-center interval of 6.5 mm with a resolution of 4.0 mm full width at half maximum in the transaxial plane. The PET images were contrasted to the standard magnetic resonance imaging (MRI) of the cat brain prepared by our laboratory,<sup>32</sup> which had been acquired at the positions in a cat brain atlas and had been reoriented to match the PET

**Table 1** Tissue distribution of radioactivity in mice after intravenous injection of [<sup>11</sup>C]SA4503

	% Injection dose/g tissue*					
	1 min	5 min	15 min	30 min	60 min	90 min
Blood	1.49 ± 0.12	0.68 ± 0.11	0.46 ± 0.07	0.29 ± 0.04	0.31 ± 0.05	0.39 ± 0.08
Brain	2.22 ± 0.52	3.61 ± 0.39	3.54 ± 0.38	3.36 ± 0.26	3.10 ± 0.23	2.79 ± 0.35
Heart	5.98 ± 1.11	3.12 ± 0.41	1.39 ± 0.11	0.75 ± 0.05	0.75 ± 0.08	0.59 ± 0.17
Lung	28.5 ± 11.6	16.0 ± 1.57	8.60 ± 1.15	4.04 ± 0.52	3.16 ± 0.21	2.27 ± 0.14
Liver	4.20 ± 0.62	7.72 ± 0.70	12.3 ± 1.16	11.9 ± 1.07	13.6 ± 1.33	8.68 ± 1.34
Pancreas	3.96 ± 0.94	7.86 ± 0.60	10.7 ± 1.35	12.3 ± 1.17	14.0 ± 0.88	15.7 ± 2.67
Spleen	2.11 ± 1.38	5.04 ± 0.67	6.16 ± 0.29	5.59 ± 0.64	4.99 ± 0.17	4.58 ± 0.86
Kidney	9.71 ± 1.10	10.5 ± 1.46	6.60 ± 0.51	4.36 ± 0.28	3.34 ± 0.15	2.37 ± 0.42
Testis	0.54 ± 0.13	0.90 ± 0.10	1.22 ± 0.06	1.25 ± 0.11	1.68 ± 0.36	2.00 ± 0.39
Muscle	1.81 ± 0.36	1.53 ± 0.15	0.97 ± 0.16	0.69 ± 0.08	0.45 ± 0.06	0.55 ± 0.13
Bone	1.43 ± 0.63	1.82 ± 0.22	1.85 ± 0.17	1.71 ± 0.09	1.35 ± 0.04	1.89 ± 0.41

\*Mean ± S.D. (n = 4)

**Table 2** Tissue distribution of radioactivity in mice after intravenous injection of [<sup>11</sup>C]SA4503

	% Injection dose/organ*					
	1 min	5 min	15 min	30 min	60 min	90 min
Brain	1.04 ± 0.27	1.73 ± 0.20	1.64 ± 0.13	1.52 ± 0.13	1.44 ± 0.13	1.25 ± 0.18
Heart	0.90 ± 0.16	0.50 ± 0.06	0.22 ± 0.01	0.15 ± 0.03	0.11 ± 0.01	0.091 ± 0.024
Lung	6.58 ± 1.78	3.68 ± 0.49	1.82 ± 0.16	0.96 ± 0.09	0.74 ± 0.05	0.53 ± 0.02
Liver	8.11 ± 0.68	14.9 ± 1.50	24.4 ± 3.29	20.1 ± 2.65	25.9 ± 0.66	16.2 ± 3.91
Pancreas	0.67 ± 0.18	1.32 ± 0.21	1.71 ± 0.23	2.10 ± 0.23	2.27 ± 0.26	2.48 ± 0.29
Spleen	0.29 ± 0.12	0.84 ± 0.25	0.90 ± 0.11	0.75 ± 0.26	0.78 ± 0.18	0.56 ± 0.08
Stomach	1.16 ± 0.38	1.71 ± 0.14	2.10 ± 0.40	1.57 ± 0.34	1.44 ± 0.06	1.34 ± 0.23
S. Intestine	4.80 ± 0.76	6.64 ± 0.48	8.70 ± 0.31	8.18 ± 1.32	11.5 ± 0.90	10.0 ± 0.90
L. Intestine	1.11 ± 0.06	1.89 ± 0.41	2.18 ± 0.13	2.79 ± 0.22	2.82 ± 0.42	2.97 ± 0.48
Kidney	5.42 ± 1.22	5.91 ± 0.88	3.66 ± 0.37	2.73 ± 0.31	1.89 ± 0.18	1.29 ± 0.22
Testis	0.11 ± 0.04	0.18 ± 0.01	0.21 ± 0.08	0.24 ± 0.04	0.29 ± 0.05	0.38 ± 0.05
Bladder	0.042 ± 0.017	0.047 ± 0.010	0.057 ± 0.021	0.078 ± 0.030	0.11 ± 0.04	0.12 ± 0.04
Urine	0.11	0.31	0.75	0.84	2.80	2.20

\*Mean ± S.D. (n = 4)

images. Major intracranial structures were identified on the PET images by referring to the MRI and to the atlas,<sup>29,35</sup> and regions of interest (ROIs) were placed over the cortex and cerebellum, and regional time-activity curves were obtained for each scan as described.<sup>28</sup> Decay-corrected radioactivity was expressed as a percentage of the injected dose per mL tissue volume (%ID/mL).

Blood was collected from the femoral artery at 1, 5, 10, 15, 20, 30, 45, 60 and 90 min after the tracer injection. The radioactivity level of the plasma was assessed as the %ID/mL and the labeled metabolites were analyzed by HPLC as previously described.<sup>15</sup>

## RESULTS

The tissue distribution of the radioactivity after injection of [<sup>11</sup>C]SA4503 into mice is summarized in Tables 1 and 2. The lung showed the highest initial uptake followed by a gradual decrease. The mean radioactivity level in the blood, heart and muscle gradually decreased. The uptake by the pancreas and testis gradually increased over 90

min. In the spleen and bone, uptake increased for the first 15 min and then decreased until 90 min. In the liver, uptake increased for the first 60 min and then decreased until 90 min. The brain uptake was increased for the first 5 min and was gradually decreased until 90 min.

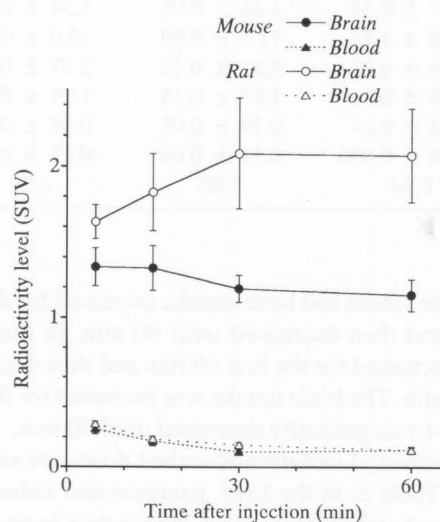
The estimated radiation-absorbed doses are summarized in Table 3. In the liver, pancreas and kidney, the radiation-absorbed doses were higher than in the other organs studied.

Figure 2 shows the time radioactivity curves expressed as the SUV of the mouse brain in the present study and of the rat brain in a previous study.<sup>15</sup> The brain uptake gradually decreased in mice, but gradually increased in rats.

The effects of the carrier dose on the brain uptake of [<sup>11</sup>C]SA4503 at 30 min after tracer injection are summarized in Figure 3. The brain uptake was significantly decreased at a dose of 70 nmol/kg and over. At a dose of 2000 nmol/kg, the brain uptake was reduced to approximately 30% of the control. The radioactivity level in the blood slightly increased with the dose.

**Table 3** Absorbed dose of [ $^{11}\text{C}$ ]SA4503 for human adults estimated from mouse data

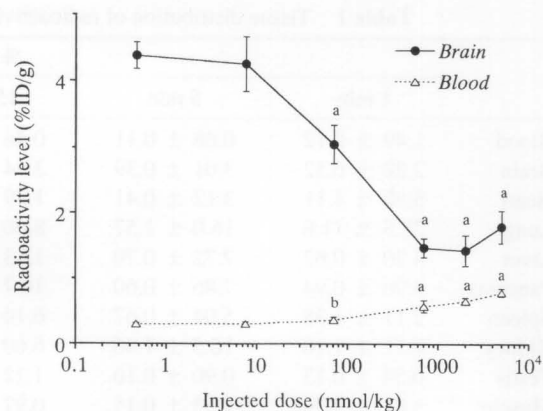
	$\mu\text{Gy}/\text{MBq}$
Brain	1.63
Thyroid	2.52
Thymus	2.86
Breast	2.48
Heart	1.01
Lungs	4.19
Livers	20.5
Pancreas	19.9
Spleen	9.63
Stomach wall	8.61
Small intestine wall	14.6
Upper large intestine wall	10.9
Lower large intestine wall	10.3
Adrenals	4.92
Kidneys	19.1
Testis	8.50
Ovaries	3.75
Uterus	3.65
Bladder	2.51
Bone surfaces	2.90
Red marrow	2.49
Bones	17.1
Total body	8.56



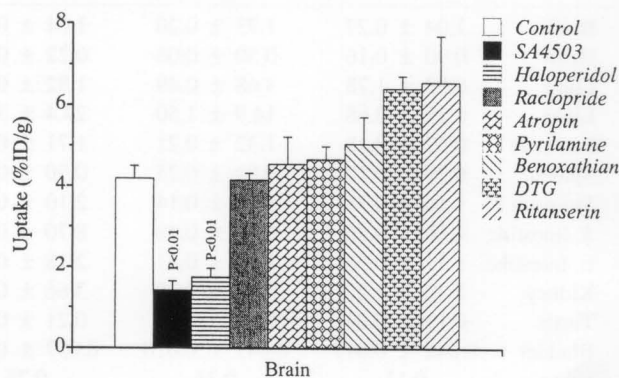
**Fig. 2** Time-radioactivity curves of the brain and plasma after intravenous injection of [ $^{11}\text{C}$ ]SA4503 into mice and rat. Time-radioactivity curves of rat brain and plasma was quoted from a previously published article.<sup>15</sup> The level of the radioactivity was expressed as the standardized uptake value (SUV). Mean  $\pm$  S.D. ( $n = 4$ ).

Figure 4 shows the effects of the co-injection of one of the eight receptor ligands or cold SA4503 on the brain uptake of [ $^{11}\text{C}$ ]SA4503. The brain uptake was reduced to approximately 30–40% of the control by SA4503 or haloperidol, but not by any of the other six ligands.

Two PET scans were performed on the same indi-



**Fig. 3** Effects of carrier-loading on the brain uptake of radioactivity at 30 min after intravenous injection of [ $^{11}\text{C}$ ]SA4503 into mice. Injected dose of [ $^{11}\text{C}$ ]SA4503 and cold SA4503 was 0.46 nmol/kg (1.1 MBq/16 pmol) and 7, 70, 700, 2000 and 5000 nmol/kg, respectively. Mean  $\pm$  S.D. ( $n = 5$ ). <sup>a</sup> $p < 0.01$  and <sup>b</sup> $p < 0.05$ : Mann-Whitney U-test compared with the control.

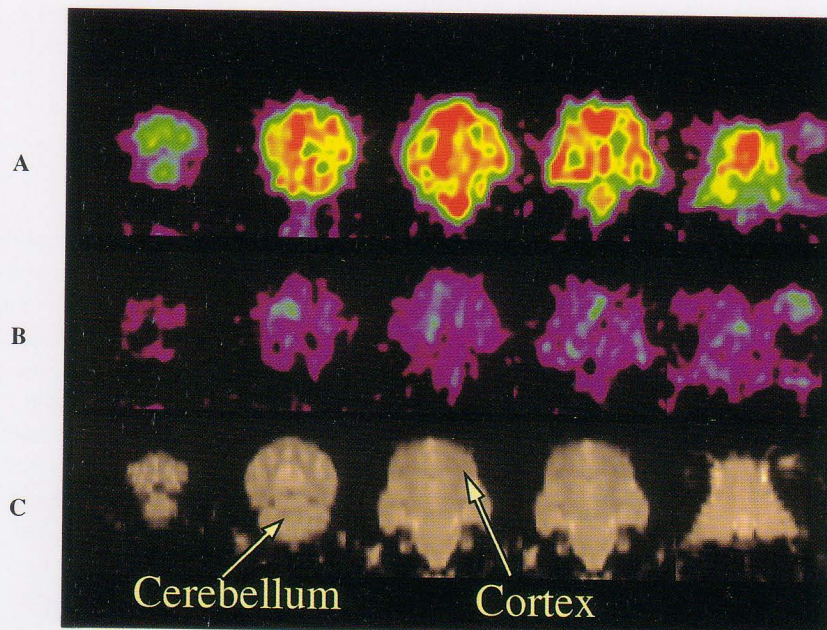


**Fig. 4** Effects of carrier-loading on the brain uptake of radioactivity at 30 min after intravenous injection of [ $^{11}\text{C}$ ]SA4503 into mice. Injected dose of [ $^{11}\text{C}$ ]SA4503 was 2.0 MBq/15 pmol and co-injected dose of each receptor ligand was 2000 nmol/kg. Mean  $\pm$  S.D. ( $n = 4$ ).  $p < 0.01$ : Mann-Whitney U-test compared with the control. DTG, 1,3-di[2-tolyl]guanidine.

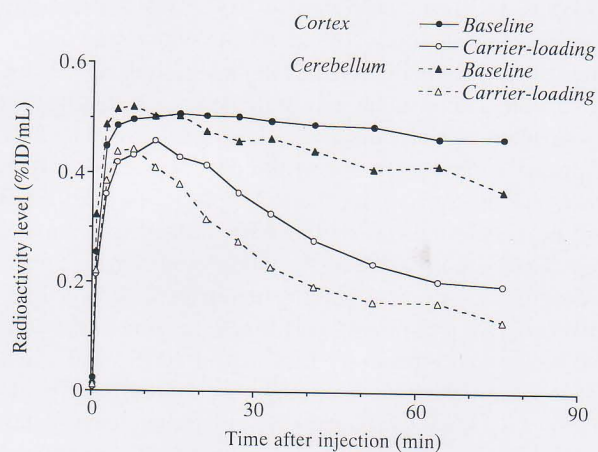
vidual: baseline measurement and carrier-loading condition. In the baseline measurement, a high uptake of radioactivity was observed in the cortex (Fig. 5A). The time-radioactivity curves for the cortex and cerebellum are shown in Figure 6. In the baseline measurement, the radioactivity increased for the first 10 min and then gradually decreased until 90 min. In the carrier-loading condition, the brain radioactivity disappeared (Fig. 5B). The radioactivity in the cortex and the cerebellum rapidly decreased after the initial uptake (Fig. 6). The radioactivity levels in the cortex were 67%, 47% and 41% of the baseline at 30 min, 60 min and 90 min, respectively, after the tracer injection. In the cerebellum, the radioactivity levels were 53%, 39% and 35% of the baseline at 30 min, 60 min and 90 min, respectively, after the tracer injection.

Figure 7 shows time-activity curves of blood and plasma.

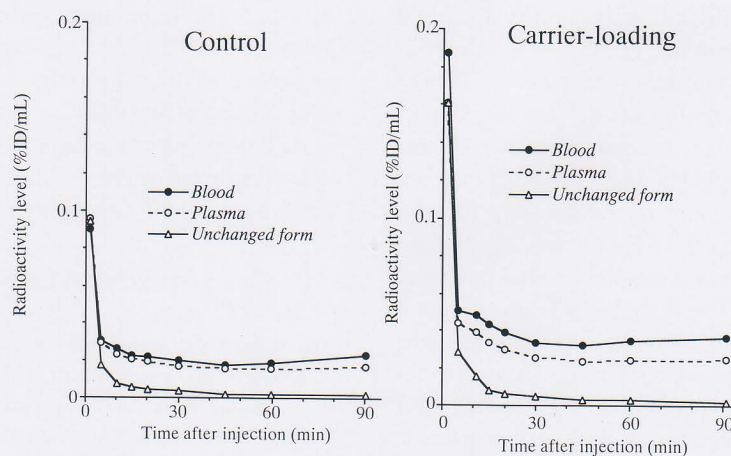




**Fig. 5** Images of the cat brain by PET with [ $^{11}\text{C}$ ]SA4503 (A) and [ $^{11}\text{C}$ ]SA4503 with carrier SA4503 (1 mg/kg) (B) and a standard MRI of the corresponding slices (C). The PET images were acquired from 60 to 80 min after injection. The range of radioactivity levels were 0.45–0.10 (percentage of injected dose per mL tissue volume, %ID/mL).



**Fig. 6** Time-radioactivity curves of the cortex and cerebellum of the cat after intravenous injection of [ $^{11}\text{C}$ ]SA4503 in the baseline and carrier loading studies. The radioactivity levels are expressed as the percentage of injected dose per mL tissue volume (%ID/mL).



**Fig. 7** Time-radioactivity curves of plasma and blood of the cat after intravenous injection of [ $^{11}\text{C}$ ]SA4503 in the baseline and carrier loading studies. The radioactivity levels are expressed as the percentage of injected dose per gram tissue volume (%ID/g).

The radioactivity rapidly decreased and was higher in blood than in plasma for 90 min after tracer injection. The time course of unchanged [ $^{11}\text{C}$ ]SA4503 in plasma is also shown in Figure 7. In the baseline measurement and carrier-loading condition, percentages of unchanged [ $^{11}\text{C}$ ]SA4503 rapidly decreased: 26.3% at 15 min, 21.9% at 30 min, 12.5% at 60 min and 11.0% at 90 min in the baseline measurement; and 24.0% at 15 min, 19.4% at 30 min, 13.1% at 60 min and 7.3% at 90 min in the carrier-loading condition.

## DISCUSSION

In the previous *in vivo* study on rats, we demonstrated that [ $^{11}\text{C}$ ]SA4503 has the potential for mapping sigma $_1$  receptors in the CNS as a PET ligand.<sup>15</sup> The high receptor-specific uptake of [ $^{11}\text{C}$ ]SA4503 by the brain was confirmed by competition with cold SA4503 and haloperidol, a representative sigma receptor ligand. The previous *in vitro* study has shown that SA4503 has a selective affinity for sigma $_1$  receptors, and has over 100 times weaker affinities for sigma $_2$ ,  $\alpha_1$ -adrenargic, dopamine D $_2$ , 5-HT $_{1A}$ , 5-HT $_2$ , histamine H $_1$ , muscarinic M $_1$  and muscarinic M $_2$  receptors.<sup>19,20</sup> In the present *in vivo* study on mice, we confirmed that [ $^{11}\text{C}$ ]SA4503 did not bind to these receptors (Fig. 4). On the other hand, as shown in Figure 4, the brain uptake of [ $^{11}\text{C}$ ]SA4503 was reduced by co-injection of haloperidol, suggesting that approximately 70% of the radioactivity in the mouse brain reflects the sigma $_1$  receptor-mediated uptake. The brain uptake of [ $^{11}\text{C}$ ]SA4503 was not saturated up to 7 nmol/kg body weight of the injected dose (Fig. 3). In the present study, it is not clear whether [ $^{11}\text{C}$ ]SA4503 is bound to sigma $_2$  receptors. Because no selective sigma $_2$  ligand with a high affinity is commercially available, DTG was used as a blocker. DTG which has relatively low affinity for sigma $_1$  (IC $_{50}$  = 246 nM) and sigma $_2$  (IC $_{50}$  = 362 nM) receptors compared with SA4503,<sup>20</sup> did not reduce but rather enhanced the brain uptake of [ $^{11}\text{C}$ ]SA4503, possibly due to low penetration across the blood-brain barrier and/or the pharmacological effect. The co-injected dose of DTG (1600 nmol/kg) was close to the lethal dose.

The present study evaluated the radiation absorbed dose of [ $^{11}\text{C}$ ]SA4503 in humans. The radiation absorbed-dose was higher in the liver, kidney and pancreas than in other organs studied, but was low enough for clinical use.

The PET study of the cat brain demonstrated the saturable binding of [ $^{11}\text{C}$ ]SA4503. A high density of radioactivity was observed in the cortex. Approximately 50–60% of the radioactivity reflects the receptor-specific binding in the cortex and cerebellum at 60–80 min after injection. In a previous *ex vivo* ARG study of the rat brain, we found a high uptake of [ $^{11}\text{C}$ ]SA4503 in the vestibular nucleus, temporal cortex, cingulate cortex, inferior colliculus, thalamus and frontal cortex, and confirmed 75–85% of the total radioactivity to be the receptor-specific at 30 min after the

tracer injection.<sup>15</sup> The brain uptake of [ $^{11}\text{C}$ ]SA4503 in both rats<sup>15</sup> and mice was blocked to a similar degree by a sigma $_1$  receptor ligand haloperidol (IC $_{50}$  = 3 nM for sigma $_1$  and IC $_{50}$  = 120 nM for sigma $_2$ )<sup>20</sup> and non-radioactive SA4503. Therefore, the saturable binding sites of [ $^{11}\text{C}$ ]SA4503 measured in the cat brain by PET may reflect sigma $_1$  receptors.

An *in vitro* study suggested that there was a species difference for sigma receptors: the concentration of sigma $_1$  receptors was slightly higher in the guinea pig brain than in the rat brain.<sup>30</sup> In the present *in vivo* study, a slight species difference was found in the time-radioactivity curves of the brain (Figs. 2 and 6). The brain uptake measured by tissue dissection gradually decreased for 90 min in mice, but gradually increased in rats. In the cat measured by PET, the brain uptake increased for the first 10 min and then gradually decreased. These results may reflect the different affinity of the tracer for sigma $_1$  receptors on the brain of the three species, but, regarding specific binding, no species difference was found. The specific binding of [ $^{11}\text{C}$ ]SA4503 in the cat brain measured by PET was estimated to be approximately 60% of the total uptake at 60–80 min after injection, which was slightly lower than that in the rat brain (approximately 70% at 30 min after injection with tissue dissection and 75–85% at 30 min with *ex vivo* ARG) or that in the mouse brain (approximately 70% at 30 min with tissue dissection).<sup>15</sup> The slight discrepancy in these results may be due to a technical problem, not to the species difference. In general, the activity on small ROIs on measured by PET appears to be lower due to the partial volume effect, whereas the activity on the ROIs placed on autoradiograms is determined exactly. With tissue dissection, the uptake of [ $^{11}\text{C}$ ]SA4503 by the whole brain, where sigma $_1$  receptors are present heterogeneously, was measured. Recently we preliminary performed imaging of the conscious monkey brain by PET with [ $^{11}\text{C}$ ]SA4503, and found that the specific binding was approximately 50–60% of the total uptake at 60–80 min (unpublished data). A clear species difference was observed in the peripheral metabolism of [ $^{11}\text{C}$ ]SA4503. In the rat, metabolic change seemed to be slow: approximately 80% of the radioactivity was found in the unchanged form in plasma 30 min postinjection<sup>15</sup> but the unchanged form of [ $^{11}\text{C}$ ]SA4503 in plasma of the cat rapidly decreased: 21.9% of total radioactivity at 30 min, 12.5% at 60 min and 11.0% at 90 min in the baseline measurement.

In conclusion, we have demonstrated that [ $^{11}\text{C}$ ]SA4503 has the potential for mapping sigma $_1$  receptors by PET.

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