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A feasibility study of [^{11}C]SA4503-PET for evaluating sigma$_1$ receptor occupancy by neuroleptics: the binding of haloperidol to sigma$_1$ and dopamine D$_2$-like receptors

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We investigated feasibility of positron emission tomography (PET) with [^{11}C]SA4503 for evaluating the sigma$_1$ receptor occupancy rate by neuroleptics. Haloperidol, which is well known to bind dopamine D$_2$-like receptor (D2R) as well as to be a representative non-selective antagonist for sigma$_1$ receptor (σ1R), was selected as a model drug. Three healthy male subjects underwent 60-min [^{11}C]raclopride-PET and 90-min [^{11}C]SA4503-PET scans successively at a 120-min interval twice in a day for baseline measurement and on another day for haloperidol-loading measurement 16 hours after peroral administration of 3 mg of haloperidol. Binding potential (BP) of [^{11}C]raclopride and [^{11}C]SA4503 was quantitatively evaluated and the σ1R and D2R occupancy rates were determined. D2R occupancy rates by haloperidol were 64% and 62% in the caudate and putamen, respectively, 16 h after the administration, while σ1R occupancy rates were approximately 80% in all seven regions investigated including the caudate, putamen and cerebellum 18 h after the administration, suggesting that the σ1R receptor occupancy rate by haloperidol was slightly larger than the D2R receptor occupancy rate. We concluded that [^{11}C]SA4503-PET can be used for evaluating the σ1R occupancy rates by neuroleptics or other drugs.

Key words: [^{11}C]SA4503, sigma$_1$ receptor, receptor occupancy, haloperidol, positron emission tomography

INTRODUCTION

In vivo evaluation of receptor occupancy by antipsychotic and antihistamineric drugs in the human brain has been investigated extensively by positron emission tomography (PET) and single-photon emission computed tomography (SPECT) with appropriate radioligands.[1,2] These *in vivo* techniques are very useful for evaluating the therapeutic effects of the drugs, for determining appropriate dosages and for developing new drugs. So far, the occupancy of drugs for dopamine, serotonin and histamine receptors were mainly evaluated.[1–3] It is also well known that a number of neuroleptics possess moderate to high affinity for sigma binding sites, suggesting the possibility that sigma receptors mediate some of the antipsychotic effects of neuroleptics.[4,5] The physiological and pathophysiological roles of the sigma receptors remain under investigation and are considered as targets of pharmaceuticals for several diseases.[6] However, the sigma receptor occupancy rates by the therapeutic drugs have not been evaluated in humans by PET or SPECT, because no *in vivo* selective radioligand was available. Recently,

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Table 1 Binding potential (BP) of [^{11}C]SA4503 and [^{11}C]raclopride in the baseline and haloperidol-loading conditions and receptor occupancy rates by haloperidol

	[^{11}C]SA4503			[^{11}C]Raclopride		
	Binding potential*		Sigma$_1$ receptor occupancy	Binding potential*		Dopamine D_2 receptor occupancy
	Baseline	Haloperidol	%	Baseline	Haloperidol	%
Caudate	16.0 ± 1.4	3.0 ± 0.5	81.2 ± 4.9	3.3 ± 0.5	1.2 ± 0.3	64.4 ± 8.9
Putamen	18.5 ± 3.2	3.2 ± 0.5	81.9 ± 6.2	3.9 ± 0.6	1.5 ± 0.3	62.3 ± 7.8
Cerebellum	29.0 ± 1.9	5.7 ± 0.7	80.4 ± 3.1			
Frontal lobe	20.8 ± 0.8	3.8 ± 0.8	81.5 ± 3.9			
Temporal lobe	26.1 ± 4.1	4.7 ± 1.2	81.4 ± 7.0			
Occipital lobe	20.3 ± 2.1	3.9 ± 1.3	81.1 ± 4.7			
Thalamus	20.9 ± 2.1	4.8 ± 0.7	76.9 ± 2.7			

Data show mean ± SD (n = 3). *Binding potential was evaluated based on a 2-tissue 3-compartment model for [^{11}C]SA4503[9] and a reference tissue model for [^{11}C]raclopride.[15]

we have developed [^{11}C]SA4503 as a selective PET ligand for sigma$_1$ receptor (σ1R),[7–9] and clinically applied it to measuring σ1Rs of patients with Alzheimer's and Parkinson's disease.[6,10]

Previously, we investigated in mice using a tissue dissection technique whether [^{11}C]SA4503 is available as an *in vivo* probe for evaluating the σ1R occupancy rates by neuroleptics using PET.[11] We selected haloperidol and two other dopamine D_2-like receptor (D2R) ligands which had high affinity for D2Rs and different affinity for sigma receptors. In the present study, we measured the σ1R occupancy rate by haloperidol in the human brain by [^{11}C]SA4503-PET as a feasibility study that [^{11}C]SA4503-PET can be applied to evaluating σ1R occupancy rates by therapeutics. We also performed [^{11}C]raclopride-PET in the same subjects for evaluating D2R occupancy rates by haloperidol.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Ethical Committee. Three male volunteers (24 ± 4 years old) who were healthy according to the history and clinical investigations and showed no abnormality on brain MRI participated in the present study, with written informed consent obtained from each. All subjects underwent two PET scans with [^{11}C]raclopride and [^{11}C]SA4503 twice on two separate days: first for baseline measurement and 2–6 weeks later for haloperidol-loading conditions. On each day [^{11}C]raclopride-PET was started late morning and two hours later [^{11}C]SA4503-PET was followed, because [^{11}C]raclopride shows a faster clearance rate from the brain than [^{11}C]SA4503. On the second day the subjects were perorally given 3 mg of haloperidol 16 hours before [^{11}C]raclopride-PET.

The PET camera used was SET-2400W (Shimadzu, Kyoto, Japan), which has an axial field-of-view of 20 cm and acquires 63 slices at a center-to-center interval of 3.125 mm.[12] The injected doses of [^{11}C]raclopride[13] were 336 ± 17 MBq/5.6 ± 2.9 nmol (specific activity 74 ± 36 TBq/mmol) and those of [^{11}C]SA4503[7] were 512 ± 160 MBq/13.6 ± 9.3 nmol (specific activity 51 ± 26 TBq/mmol). After transmission scanning with a rotating [^{68}Ge]/[^{68}Ga] line source to correct for attenuation, [^{11}C]raclopride was intravenously injected into the subject, and then a 60-min PET scanning in 2D mode (10 sec × 6 frames, 30 sec × 3 frames, 60 sec × 5 frames, 150 sec × 5 frames, and 300 sec × 8 frames) was performed without arterial blood sampling. In the second PET scan with [^{11}C]SA4503, a 90-min dynamic scan in 2D mode (10 sec × 6 frames, 30 sec × 3 frames, 60 sec × 5 frames, 150 sec × 5 frames, and 300 sec × 14 frames) was carried out together with arterial blood sampling at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 135 and 150 sec and 3, 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 70, 80 and 90 min. The radioactivity levels in plasma were measured for radioactivity for gamma-counter and the time-activity curve (TAC) of plasma was calculated as Bq/m*l* and SUV. Metabolites of [^{11}C]SA4503 in the plasma sampled at 3, 10, 20, 30, 40 and 60 min were analyzed by high-performance liquid chromatography as previously described.[7]

The tomographic images were reconstructed using a Fourier rebinning algorithm[14] and a filtered backprojection method with Butterworth filter (cutoff frequency 1.25 cycle/cm and order 2). The data were collected in a 128 × 128 × 63 matrix, and the voxel size was 2 × 2 × 3.125 mm. Voxel counts were calibrated to activity concentration (Bq/m*l*). Regions of interest (ROIs) were placed over the caudate, putamen, cerebellum, frontal lobe, temporal lobe, occipital lobe and thalamus and on the PET images with reference to MRI. TACs of these ROIs were calculated as Bq/m*l* and SUV. For quantitative measurement of the radioligand-receptor binding, the binding potential (BP) of [^{11}C]raclopride was calculated by the RPM with the

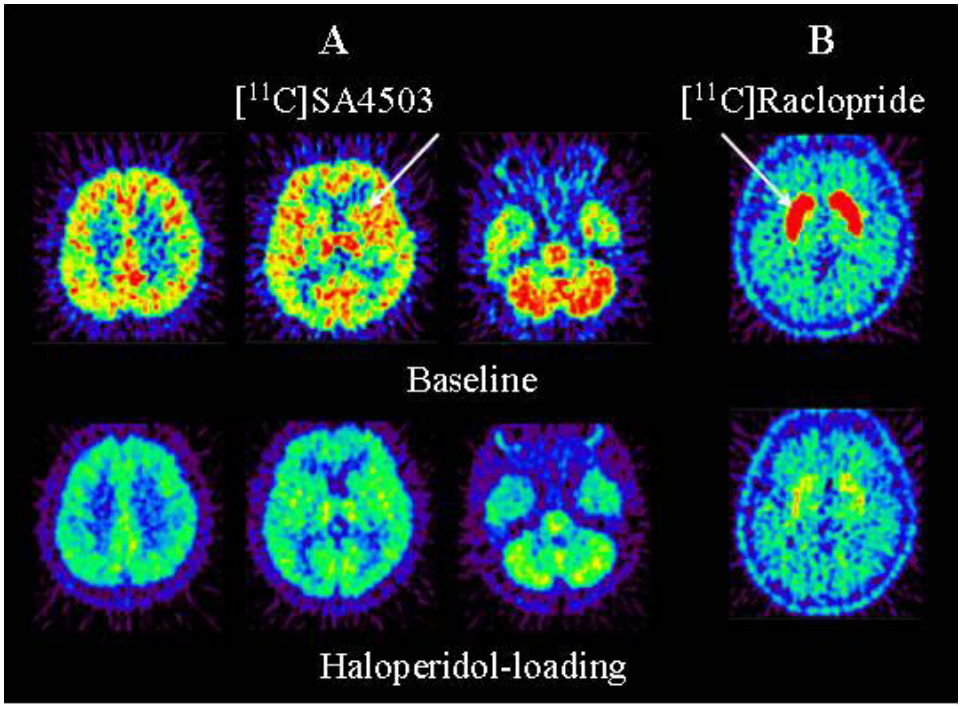

Fig. 1 PET images of [^{11}C]SA4503 (A) and [^{11}C]raclopride (B) in the same healthy subject in the baseline (*upper images*) and haloperidol-loading (*lower images*) conditions. The images of [^{11}C]SA4503 and [^{11}C]raclopride were acquired for 60–90 min and 40–60 min, respectively. White arrows show the striatum.

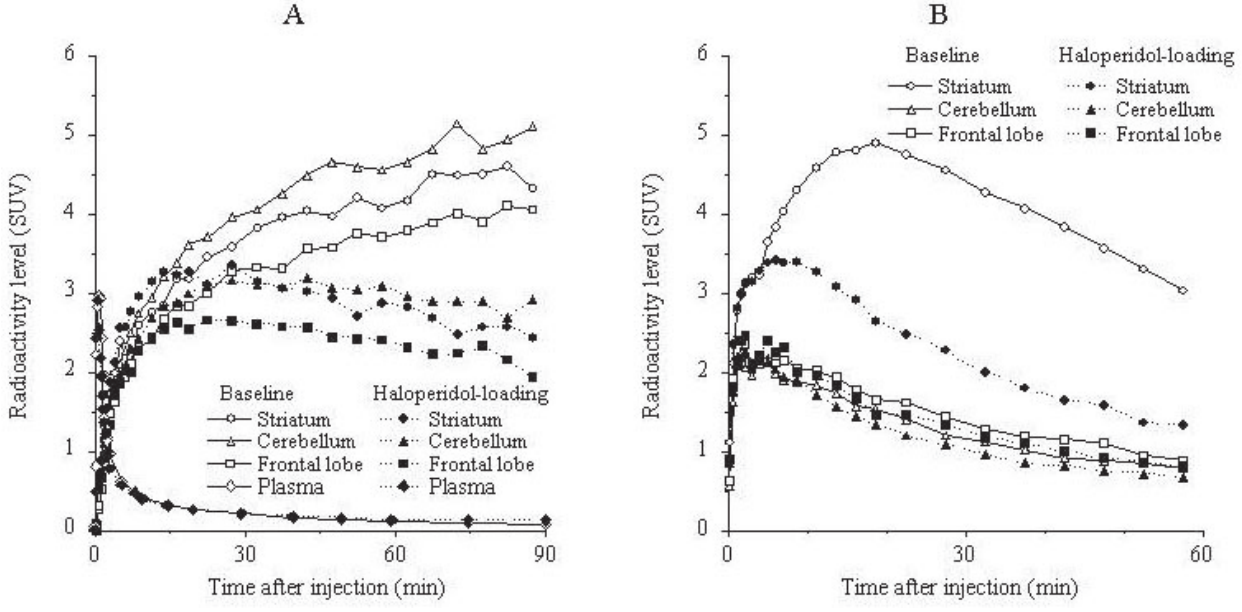

Fig. 2 Time-activity curves course of [^{11}C]SA4503 (A) and [^{11}C]raclopride (B) in the human brain and plasma in the baseline and haloperidol-loading conditions. Three regions of the brain were representatively selected: striatum (including caudate and putamen), cerebellum and frontal lobe.

cerebellum as a reference region.[15] For the binding of [^{11}C]SA4503, using the TACs of tissues and the metabolite-corrected TAC of plasma, the kinetic analysis was carried out based on a 2-tissue 3-compartment model having 4 parameters: K_1, influx rate constant from plasma to brain tissue; k_2, efflux rate constant from tissue to plasma; k_3, association rate constant between [^{11}C]SA4503 and sigma$_1$ receptor; and k_4, dissociation rate constant of

[^{11}C]SA4503-receptor complex.[9] The BP was expressed as k_3/k_4. The detailed validation of kinetic analysis of [^{11}C]SA4503 will be described elsewhere. The receptor occupancy (%) was defined as 100 × [(BP in baseline) – (BP in haloperidol-loading)]/(BP in baseline).

RESULTS AND DISCUSSION

Static PET images of [^{11}C]SA4503 and [^{11}C]raclopride in the baseline and haloperidol-loading conditions are represented in Figure 1. In the baseline, [^{11}C]SA4503 was taken up in all regions, whereas [^{11}C]raclopride was highly concentrated in the striatum. By the haloperidol-loading, the uptake of [^{11}C]SA4503 was reduced in all brain regions, while that of [^{11}C]raclopride was blocked in the striatum. The TACs in the striatum, cerebellum, frontal lobe and plasma are represented in Figure 2. The levels of radioactivity in plasma rapidly decreased to a similar extent in the two conditions. In the baseline measurement of [^{11}C]SA4503, the levels of radioactivity increased in all seven regions of the brain over 90 min in the case of Figure 2, or reached an apparent equilibrium state for 60 to 90 min in the other case (not shown), whereas the levels reached a plateau for 15–30 min and then decreased in the haloperidol-loading. The mean SUV values of [^{11}C]SA4503 for 60–90 min in the haloperidol-loading ranged approximately 55–65% of those in the baseline in the seven regions. BP was greatly reduced and the σ1R occupancy rates by haloperidol were approximately 80% in the seven regions investigated (Table 1).

In the case of [^{11}C]raclopride, the striatal uptake was greatly reduced by haloperidol-loading, while the levels of radioactivity in the other regions were not influenced by the haloperidol-treatment. The D2R occupancy rates based on BP values were 64% and 62% in the caudate and putamen, respectively (Table 1).

A number of neuroleptics possess moderate to high affinity for sigma binding sites, suggesting the possibility that sigma receptors mediate some of the antipsychotic effects of neuroleptics.[4] Frieboes et al. reported that haloperidol and the specific sigma ligand panamesine have similar antipsychotic properties regarding immunomodulation and sleep-electroencephalographic changes.[16] These findings suggest indirectly the binding of haloperidol to sigma receptors. The present study directly demonstrated the binding of haloperidol to σ1Rs in the human brain by [^{11}C]SA4503-PET. It is notable that the σ1R occupancy rates by haloperidol were larger than the D2R occupancy rates. A similar finding was previously observed in mice using a tissue dissection technique.[11] However, the present and previous studies indicated no association between σ1R occupancy rates and antipsychotic efficacy of haloperidol. Thus, evaluation of the σ1R occupancy rates by haloperidol or other antipsychotic drugs in relation to behavioral potency in humans by [^{11}C]SA4503-PET is of great interest.

In the present study, we performed quantitative evaluation of the binding of [^{11}C]SA4503 to σ1Rs using a standard method based on a 2-tissue 3-compartment model having 4 parameters. We also applied the graphical analysis using a Logan plot to the quantitative evaluation.[17] The Logan plot is a suitable method for parametric imaging of ligand-receptor binding because of its algorithmic simplicity and fast calculation speed, but it provides total distribution volume including both specific and non-specific binding, but not BP. However, the results showed good agreement in the receptor occupancy rates between BP and DVt, and it implied that the receptor density of σ1Rs could be visualized using Logan plot. The details will be described elsewhere.

In conclusion, [^{11}C]SA4503-PET can be used for evaluating σ1R occupancy rates by neuroleptics or other drugs. The technique could be valuable for developing new drugs and for evaluating the therapeutic effects of drugs in term of σ1R occupancy.

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