Striatal and Extrastriatal Dopamine D_2 Receptor Occupancy by a Novel Antipsychotic, Blonanserin

A PET Study With [^{11}C]Raclopride and [^{11}C]FLB 457 in Schizophrenia

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Abstract: Blonanserin is a novel antipsychotic with high affinities for dopamine D_2 and 5-HT_{2A} receptors, and it was recently approved for the treatment of schizophrenia in Japan and Korea. Although double-blind clinical trials have demonstrated that blonanserin has equal efficacy to risperidone, and with a better profile especially with respect to prolactin elevation, its profile of in vivo receptor binding has not been investigated in patients with schizophrenia. Using positron emission tomography (PET), we measured striatal and extrastriatal dopamine D_2 receptor occupancy by blonanserin in 15 patients with schizophrenia treated with fixed doses of blonanserin (ie, 8, 16, and 24 mg/d) for at least 4 weeks before PET scans, and in 15 healthy volunteers. Two PET scans, 1 with [^{11}C]raclopride for the striatum and 1 with [^{11}C]FLB 457 for the temporal cortex and pituitary, were performed on the same day. Striatal dopamine D_2 receptor occupancy by blonanserin was 60.8% (3.0%) [mean (SD)] at 8 mg, 73.4% (4.9%) at 16 mg, and 79.7% (2.3%) at 24 mg. The brain/plasma concentration ratio calculated from D_2 receptor occupancy in the temporal cortex and pituitary was 3.38, indicating good blood-brain barrier permeability. This was the first study to show clinical daily dose amounts of blonanserin occupying dopamine D_2 receptors in patients with schizophrenia. The clinical implications obtained in this study were the optimal therapeutic dose range of 12.9 to 22.1 mg/d of blonanserin required for 70% to 80% dopamine D_2 receptor occupancy in the striatum, and the good blood-brain barrier permeability that suggested a relatively lower risk of hyperprolactinemia.

Key Words: schizophrenia, blonanserin, dopamine D_2 receptor occupancy, positron emission tomography, hyperprolactinemia

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2-(4-Ethyl-1-piperazinyl)-4-(4-fluorophenyl)-5,6,7,8,9,10-hexahydrocycloocta [*b*] pyridine, blonanserin, was developed as a novel antipsychotic drug for schizophrenia in Japan and Korea.[1–3] Blonanserin is a relatively new atypical antipsychotic with very high binding affinity for $D_{2,3}$ and 5-HT_{2A} receptors.[4,5] However, unlike some other atypical antipsychotics, blonanserin has a low affinity for other neurotransmitter receptors, including H_1, muscarinic M_1, and α1 adrenergic receptors, which may work to minimize potential adverse effects such as weight gain/sedation, dry mouth, and orthostatic hypotension, respectively. Double-blind clinical trials demonstrated that blonanserin is equal to haloperidol and risperidone about primary end points, and is better than risperidone with respect to a lower risk of prolactin elevation.[6,7]

Neuroimaging studies of dopamine receptor occupancy using positron emission tomography (PET) have elucidated the correlation between dopamine D_2 receptor occupancy and optimal dose of antipsychotic drugs (ie, sufficient antipsychotic effect and lower incidence of adverse effects).[8,9] PET studies investigating different antipsychotic drugs indicated that approximately 70% to 80% dopamine D_2 receptor occupancy in the striatum was required for antipsychotic response, and that occupancy above this range led to extrapyramidal adverse effects.[10–12] The recent systematic review of the association between dopamine D_2 receptor occupancy and clinical effects supported the presence of a therapeutic window, suggesting that a continuing occupancy-response relationship also may exist within this window (60%–78% D_2 occupancy).[13] The clinically approved daily dose of blonanserin has been settled at 8 to 24 mg based on the results of clinical trials. However, the in vivo profile of receptor binding of blonanserin in patients with schizophrenia has not been investigated, and it has not been clarified whether its clinically approved daily dose occupied D_2 receptors in line with the suggested therapeutic window.

Although some antipsychotic drugs have a risk for drug-induced hyperprolactinemia,[14] one of the beneficial characteristics of blonanserin is the lower incidence of hyperprolactinemia.[6] We recently demonstrated that the brain/plasma concentration ratio (B/P ratio) calculated from the dopamine D_2 receptor occupancies in the extrastriatal and pituitary regions reflects the permeability of the blood-brain barrier (BBB), and that it represents a good biomarker for the risk of antipsychotic-induced hyperprolactinemia.[8]

In this study, we investigated (1) the striatal and extrastriatal dopamine D_2 receptor occupancy by the clinically approved daily dose of blonanserin and (2) the B/P ratio of blonanserin to determine the BBB permeability in patients with schizophrenia using PET.

MATERIALS AND METHODS

Subjects and Study Protocol

Fifteen patients diagnosed with schizophrenia according to the *Diagnostic and Statistical Manual of Mental Disorders,*

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Fourth Edition criteria, and 15 healthy volunteers comparable to the patients in age and sex participated in the study. This study was conducted as part of an open-label postmarketing surveillance study of blonanserin in Japan (D4901439; Dainippon Sumitomo Pharma Co, Ltd), and was approved by the ethics committee and review board of Nippon Medical School Hospital, Tokyo, Japan. After complete explanation of the study, written informed consent was obtained from all participants.

Exclusion criteria were the following: (1) subjects treated with electroconvulsive therapy within 90 days before screening; (2) subjects unable to cease anti-Parkinson medication or under the influence of central nervous system depressants; (3) subjects with current or history of severe physical condition, substance abuse, suicide attempt, or suicidal ideation; (4) pregnant or potential pregnancy; and (5) subjects taking other investigational new drugs or clinical trial medicine of postmarketing surveillance. Subject age was limited to between 20 and 64 years.

Inclusion criteria were as follows: (1) subjects were treated with only blonanserin (ie, no other antipsychotic medications) for at least 4 weeks before the study; (2) subjects were treated at the same dosage of 8, 16, or 24 mg/d of blonanserin for at least 2 weeks before the screening; (3) patients took blonanserin twice a day after meals in the morning and evening; and (4) subjects scored less than 120 on the positive and negative syndrome scale (PANSS[15]) at screening.

The use of the following drugs was prohibited from the time of screening to the end of the clinical trial: (1) any other antipsychotics, (2) carbamazepine and methamphetamine hydrochloride, (3) any other drugs affecting digestive organs with dopamine D_2 receptor blocking action, (4) epinephrine, (5) CYP3A4 inhibitor or revulsant, and (6) any other investigational agent or clinical trial medicine of postmarketing surveillance.

Occurrence of extrapyramidal symptoms (EPS) was assessed by the Drug-Induced Extrapyramidal Symptoms Scale (DIEPSS).[16] DIEPSS consisted of 8 symptoms of EPS (eg, parkinsonisms, akathisia, dystonia, and dyskinesia) and 1 global assessment of the severity of EPS. In this study, we considered patients with apparent EPS if the global assessment score of DIEPSS was greater than or equal to 2, or if 4 or more symptoms were present at DIEPSS. We also considered patients with apparent hyperprolactinemia defined as plasma prolactin higher than 20.0 ng/mL for men and higher than 40.0 ng/mL for women. Estimation of dopamine D_2 receptor occupancy of patients with well-controlled schizophrenia by blonanserin was scheduled between 2 and 4 weeks after the start, because administration of antipsychotic drugs for at least 6 weeks was recommended by expert consensus guidelines for judging their effectiveness,[17] and there has been the risk of failure to synthesize the radioligand. Because of this possibility of synthesis failure, the study protocol allowed scanning between 12 and 43 days after the start. During the study period, 2 PET scans per patient were performed on the same day, the first scan with [^{11}C]FLB 457 for extrastriatal dopamine D_2 receptor occupancy, and the second scan with [^{11}C]raclopride for striatal dopamine D_2 receptor occupancy. At PET scan day, patients took blonanserin after a meal. The first PET scan was done between 1 and 3 hours after taking blonanserin and the second one between 4.5 and 6.5 hours after. The signal-to-noise ratio by high-affinity radioligand is higher than by low-affinity radioligand. Because dopamine receptor density in the extrastriatal regions is considerably lower than in the striatal region, a high-affinity radioligand such as [^{11}C]FLB 457 is suitable. On the other hand, the time to reach equilibrium condition by [^{11}C]FLB 457 is too long for the half-life of [^{11}C] labeled radioligands, so high-affinity radioligands could cause underestimation of BP_{ND} values especially in regions with high dopamine D_2 receptor densities. Thus, we used different radioligands in this study, [^{11}C]raclopride for a high-density region such as the striatum, and [^{11}C]FLB 457 for a low-density extrastriatal region.[18,19]

Venous blood samples were obtained immediately before tracer injection and after each PET scan to measure the plasma concentration of blonanserin.

PET Procedure

A PET scanner system, Eminence SET-3000GCT/X (Shimadzu Corporation, Kyoto, Japan), was used to measure regional brain radioactivity. Each scan was preceded by a 4-minute transmission scan for attenuation correction using ^{137}Cs. Dynamic PET scanning was performed for 90 minutes after intravenous bolus injection of 212.2 to 249.0 MBq/1.52 (0.25) μg (patients) and 208.8 to 239.1 MBq/1.91 (0.38) μg (healthy volunteers) of [^{11}C]FLB 457. The specific radioactivity of [^{11}C]FLB 457 was 28.3 to 77.6 GBq/μmol [mean (SD), patients, 60.7 (13.6) GBq/μmol; healthy volunteers, 49.1 (11.7) GBq/μmol]. The injected mass of [^{11}C]FLB 457 was 1.25 to 2.76 μg. Dynamic PET scanning was performed for 60 minutes after intravenous bolus injection of 211.1 to 241.8 MBq/0.70 (0.28) μg (patients) and 212.0 to 238.8 MBq/0.97 (0.31) μg (healthy volunteers) of [^{11}C]raclopride. Specific radioactivity of [^{11}C]raclopride was 57.2 to 193.9 GBq/μmol [mean (SD), patients, 140.8 (37.1) GBq/μmol; healthy volunteers, 100.2 (32.8) GBq/μmol]. The injected mass of [^{11}C]raclopride was 0.43 to 1.61 μg. Magnetic resonance (MR) images of the brain were acquired with 1.5 T MR imaging, Intera 1.5 T Achieve Nova (Philips Medical Systems, Best, Netherlands). T_1-weighted MR images were obtained at 1-mm slices.

Data Analysis

All emission scans were reconstructed with a Hanning filter cutoff frequency of 0.4. Regions of interest (ROIs) were defined for the striatum ([^{11}C]raclopride), temporal cortex ([^{11}C]FLB 457), pituitary ([^{11}C]FLB 457), and cerebellar cortex ([^{11}C]raclopride and [^{11}C]FLB 457). ROIs were drawn manually on overlaid coregistered summated PET and MR images of each subject by PMOD (PMOD Technologies Ltd, Zurich, Switzerland). The average values of right and left ROIs were used for the analysis. Dopamine D_2 receptor binding was quantified using a 3-parameter simplified reference tissue model.[20,21] The cerebellum was used as reference region because of its negligible dopamine D_2 receptor density.[22] This model allows estimation of the binding potential (BP_{ND}), which was defined as $f_{ND} \times B_{max} / K_d$, where f_{ND} is the free fraction of ligand in the nondisplaceable tissue compartment, B_{max} is the receptor density, and K_d is the dissociation constant.[23]

Dopamine D_2 receptor occupancy in the striatum and temporal cortex by blonanserin was estimated by the following equation: occupancy (%) = $(BP_{base} - BP_{drug}) / BP_{base} \times 100$, where BP_{base} is BP_{ND} in the drug-free state, and BP_{drug} is BP_{ND} of patients treated with blonanserin.[24–26] In this study, mean BP_{ND} in healthy volunteers was used as BP_{base}, as BP_{ND} in the striatum measured with [^{11}C]raclopride or in the extrastriatal regions (ie, temporal cortex and pituitary) measured with [^{11}C]FLB 457 in patients is not significantly different from that in normal control.[27–29] The same PET procedure and data analysis for BP_{ND} estimation were used for normal subjects and patients. The relationship between dose or plasma concentration of blonanserin and dopamine D_2 receptor occupancy is described by the following equation: occupancy (%) = $D / (D + ED_{50}) \times 100$ or occupancy (%) = $C_{plasma} / (C_{plasma} + EC_{50}) \times 100$, where D is the dose of blonanserin, C_{plasma} is the plasma

concentration of blonanserin, ED_{50} is the dose required to achieve 50% occupancy, and EC_{50} is the plasma concentration required to attain 50% occupancy.[24–26,30] Both ED_{50} and EC_{50} reflect the affinity of antipsychotic drug for dopamine D_2 receptor. In this study, maximum occupancy was fixed at 100%, the same as in previous occupancy studies of risperidone.[26,30]

The B/P ratio was the ratio of drug concentration inside to that outside BBB. Drug concentration in the brain or plasma was calculated by occupancy and IC_{50} as described by the following equation: $C = IC_{50} / ([100 / \text{Occupancy}] - 1)$, in which C is the drug concentration in the brain or plasma. IC_{50} was the drug concentration required to induce 50% occupancy, reflecting the affinity of antipsychotic drug to dopamine D_2 receptor, and the value of IC_{50} was assumed to be the same whether the region was outside or inside BBB. Because the pituitary exists outside BBB and the temporal cortex inside BBB, the B/P ratio can be calculated by the following equation: $\text{B/P ratio} = C_{brain} / C_{pituitary} = ([100 / \text{Occupancy}_{pituitary}] - 1) / [(100 / \text{Occupancy}_{temporal}] - 1)$,[8] where C_{brain} is the drug concentration in the vicinity of receptors in the temporal cortex, $C_{pituitary}$ is that in the pituitary, $\text{Occupancy}_{pituitary}$ is the dopamine D_2 receptor occupancy in the pituitary, and $\text{Occupancy}_{temporal}$ is that in the temporal cortex. To calculate the B/P ratio of blonanserin, we used the same area under the time-activity curve (AUC) ratio method as in the previous study to measure dopamine D_2 receptor occupancy in the pituitary.[8] The AUC method does not need the assumptions that are required for the simplified reference tissue model method.[8] The equation of the AUC method was as follows: $BP_{ND} = (AUC_{region} / AUC_{cerebellum}) - 1$. The subscript "region" denotes the pituitary cortex, and an integration interval of 60 to 90 minutes was used for the calculation of AUC. Dopamine D_2 receptor occupancy in the pituitary was estimated by the same equation as for the striatum. The cerebellum was used as reference tissue, given its negligible density of dopamine D_2 receptors.[22]

Measurement of Plasma Concentration of Blonanserin

We measured plasma concentration of blonanserin in the same way as a previous study.[31] Blood samples were collected in heparinized tubes and centrifuged for 10 minutes at 3000 rpm at 4°C. Separated plasma samples were stored at −80°C until analyzed. The plasma concentration of blonanserin was determined by a validated method using high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a target lower limit of quantification of 0.01 ng/mL (JCL Bioassay Corporation, Osaka, Japan).

Statistical Analysis

Correlations between dose or plasma concentration of blonanserin and dopamine D_2 receptor occupancy in the striatum, temporal cortex, and pituitary were assessed. Correlations between striatal occupancy and age or duration of illness were also evaluated. Paired t test was performed to compare (1) dopamine D_2 receptor occupancies between the striatum and temporal cortex and (2) plasma concentrations of blonanserin between the 2 PET scans, with [^{11}C]raclopride and [^{11}C]FLB 457, respectively, in each individual subject. In all tests, a P value of <0.05 was considered statistically significant.

RESULTS

Patient characteristics are shown in Table 1. Fifteen patients [age range, 26–40 years; mean (SD), 32.8 (4.8) years; 8 males, 7 females] and 15 comparable healthy volunteers [age range, 24–54 years; mean (SD), 36.3 (8.3) years; 8 males, 7 females] participated in the study. Average duration of illness was 9.4 (5.7) years and average age at onset of schizophrenia was 23.2 (5.4) years. Average PANSS scores of all patients were 60.2 (19.2) at screening day and 60.1 (18.1) at PET scan day (Table 2). Four patients, one taking 16 mg and three 24 mg, showed EPS (Table 2). Two of 5 patients at 8 mg, 1 of 5 patients at 16 mg, and none of 5 patients at 24 mg met the criteria of hyperprolactinemia at PET scan day (Table 2).

Striatal dopamine D_2 receptor occupancy using [^{11}C]raclopride was 56.9% to 83.7% (Table 3), and mean striatal occupancies were 60.8% (3.0%) at 8 mg/d, 73.4% (4.9%) at 16 mg/d, and 79.7% (2.3%) at 24 mg/d. ED_{50} was 5.53 mg/d (r = 0.91) and EC_{50} was 0.17 ng/mL (r = 0.52; Fig. 1). Occupancy of dopamine D_2 receptor in the striatum by

TABLE 1. Patient Characteristics

Patient Number	Sex	Age, y	Duration of Illness, y	Age at Onset, y	Dose, mg/d	No. of Days (From Screening to PET Scans)
1	Male	28	0	27	8	40
2	Male	31	7	24	8	61
3	Female	26	5	21	8	214
4	Female	29	2	27	8	47
5	Male	34	8	26	8	43
6	Male	40	13	27	16	61
7	Female	31	8	22	16	181
8	Female	40	16	24	16	40
9	Male	27	8	19	16	43
10	Male	33	16	17	16	47
11	Female	38	13	24	24	133
12	Female	39	2	36	24	66
13	Male	29	14	15	24	151
14	Female	34	10	24	24	54
15	Male	34	19	15	24	42
Mean		32.8	9.4	23.2		82
SD		4.8	5.7	5.4		58.0

TABLE 2. PANSS, EPS, and Plasma Concentration of Prolactin

Patient Number	Sex	PANSS		EPS		Plasma Concentration of Prolactin, ng/mL		Hyperprolactinemia (PET Scan Day)
		Screening Day	PET Scan Day	Screening Day	PET Scan Day	Screening Day	PET Scan Day	
1	Male	34	38	(−)	(−)	18.8	14.4	(−)
2	Male	39	38	(−)	(−)	13.8	16.2	(−)
3	Female	59	59	(−)	(−)	51.1	43.9	(+)
4	Female	75	78	(−)	(−)	80.1	59.2	(+)
5	Male	48	49	(−)	(−)	22.0	10.9	(−)
6	Male	54	55	(−)	(−)	13.7	21.9	(+)
7	Female	83	83	(+)	(+)	29.5	35.5	(−)
8	Female	56	56	(−)	(−)	15.8	19.3	(−)
9	Male	93	85	(−)	(−)	14.8	9.2	(−)
10	Male	86	87	(−)	(−)	19.6	9.4	(−)
11	Female	32	33	(+)	(+)	37.8	35.5	(−)
12	Female	44	43	(−)	(−)	20.9	31.7	(−)
13	Male	72	72	(−)	(−)	7.3	10.5	(−)
14	Female	56	56	(+)	(+)	94.8	26.9	(−)
15	Male	72	69	(+)	(+)	13.2	16.9	(−)
Mean		60.2	60.1			30.2	24.1	
SD		19.2	18.1			25.8	14.6	

blonanserin, calculated from ED_{50}, was 59.1% for 8 mg and 81.3% for 24 mg.

Dopamine D_2 receptor occupancy in the temporal cortex using [^{11}C]FLB 457 was 22.6% to 83.3% (Table 3), and mean occupancies were 46.8% (14.3%) at 8 mg/d, 70.4% (9.2%) at 16 mg/d, and 69.1% (3.3%) at 24 mg/d. ED_{50} was 8.61 mg/d (r = 0.71) and EC_{50} was 0.38 ng/mL (r = 0.13; Fig. 2). The average dopamine D_2 occupancy of [^{11}C]FLB 457 was 9.2% lower (14% at 8 mg, 3% at 16 mg, 10.6% at 24 mg) than that of [^{11}C]raclopride. Although there was a significant difference in dopamine D_2 receptor occupancy between the striatum and temporal cortex at 24 mg (P = 0.002), there were no significant differences in plasma concentrations of blonanserin between the 2 scans (P = 0.50) and in dopamine D_2 receptor occupancy between the striatum and temporal cortex at 8 and 16 mg (P = 0.41 and 0.13, respectively). There was no correlation between striatal occupancy and age or duration of illness. Dopamine D_2 receptor occupancy in the pituitary using [^{11}C]FLB 457 was

TABLE 3. Dopamine D_2 Occupancy in Temporal Cortex, Striatum, and Pituitary

Patient Number	Dose, mg/d	[^{11}C]Raclopride		[^{11}C]FLB 457		
		Plasma Concentration of Blonanserin, ng/mL	Striatum Receptor Occupancy, %	Plasma Concentration of Blonanserin, ng/mL	Temporal Cortex Receptor Occupancy, %	Pituitary Receptor Occupancy, %
1	8	0.174	61.9	0.228	49.5	43.0
2	8	0.286	63.2	0.399	55.5	78.7
3	8	0.215	58.4	0.269	47.4	2.7
4	8	0.161	56.9	0.291	59.2	2.9
5	8	0.452	63.6	0.547	22.6	14.5
Mean (SD)		0.258 (0.119)	60.8 (3.0)	0.347 (0.129)	46.8 (14.3)	28.4 (32.6)
6	16	0.503	67.2	0.878	61.7	23.6
7	16	0.385	70.7	0.669	76.4	36.3
8	16	0.698	79.5	1.261	83.3	62.3
9	16	1.008	72.6	0.877	63.2	58.3
10	16	0.736	77.1	1.035	67.2	56.0
Mean (SD)		0.666 (0.239)	73.4 (4.9)	0.944 (0.220)	70.4 (9.2)	47.3 (16.6)
11	24	0.460	78.4	0.401	68.4	30.4
12	24	1.048	79.0	1.399	71.4	66.7
13	24	0.841	79.4	1.577	63.8	61.9
14	24	0.966	83.7	0.741	72.0	66.7
15	24	1.803	78.2	2.113	69.7	68.1
Mean (SD)		1.024 (0.491)	79.7 (2.3)	1.246 (0.681)	69.1 (3.3)	58.8 (16.0)

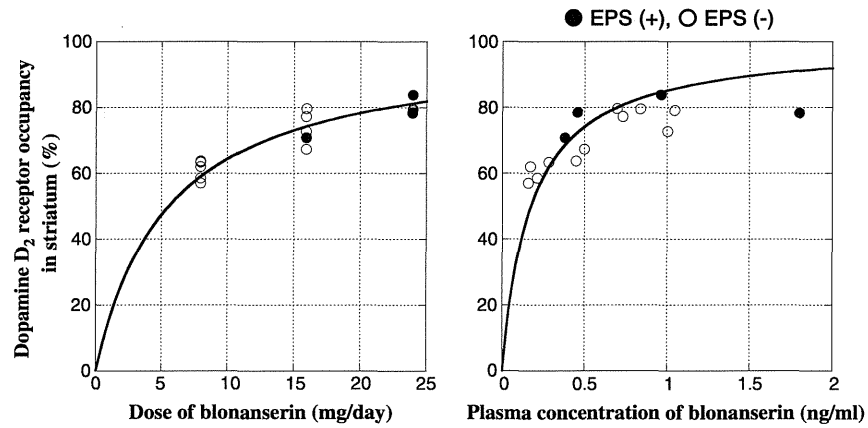

FIGURE 1. Relationship between dopamine D2 receptor occupancy in the striatum and dose or plasma concentration of blonanserin. ED_{50} in the striatum was 5.53 mg/d ($r = 0.91$) and EC_{50} was 0.17 ng/mL ($r = 0.52$) (ED_{50}, dose required to induce 50% occupancy; EC_{50}, plasma concentration required to induce 50% occupancy; EPS, extrapyramidal symptoms).

2.7% to 78.7% (Table 3), and mean occupancies were 28.4% (32.6%) at 8 mg/d, 47.3% (16.6%) at 16 mg/d, and 58.8% (16.0%) at 24 mg/d. ED_{50} was 18.06 mg/d ($r = 0.52$) and EC_{50} was 0.87 ng/mL ($r = 0.60$; Fig. 3). The B/P ratio of blonanserin calculated from our data was 3.88 (5.53).

DISCUSSION

This was the first study to investigate the dopamine D_2 receptor occupancy of the clinical daily dose of blonanserin in patients with schizophrenia. Our study demonstrated the ED_{50} value of the striatal dopamine D_2 receptor occupancy of blonanserin to be 5.53 mg/d and the EC_{50} value 0.17 ng/mL, those of the temporal cortex 8.61 mg/d and 0.38 ng/mL, and those of the pituitary 18.06 mg/d and 0.87 ng/mL. In addition, dopamine D_2 receptor occupancy of the striatum was significantly higher than that of the temporal cortex only at a blonanserin dose of 24 mg.

Before discussing the implications of this study, we should acknowledge its methodological limitation of using mean BP_{ND} in healthy volunteers to calculate the dopamine D_2 receptor occupancy, similarly to a previous study.[32] Although previous studies reported that BP_{ND} in the striatum measured with [^{11}C]raclopride or in the temporal cortex measured with [^{11}C]FLB 457 in patients is not significantly different from that in normal control,[27–29] individual differences in BP_{ND} may lead to potential error in the estimation of dopamine D_2 receptor occupancy.[10] Second, although the dosages of radioactivity of the 2 radioligands were not significantly different, their injected mass doses were significantly higher in the healthy volunteer group than in the patient group. The higher injected mass dose in our study might lower the BP in the healthy volunteers. Third, the effect of the radiolabeled metabolite of [^{11}C]FLB 457 should be considered, although a previous study indicated that a major metabolite of [^{11}C]FLB 457 had very low affinity for dopamine D_2 receptor.[33] Fourth, quantification of BP_{ND} also has methodological limitations. A previous study reported that the cerebellum could be used as a measure of nonspecific binding in the pituitary, because the fully occupied time-activity curve of the pituitary was at almost the same level as the cerebellum.[8] Therefore, we used the cerebellum as reference region in this study. However, nonspecific binding in the pituitary may not be the same as that of brain parenchyma.

We know from previous PET studies that around 70% to 80% occupancy in the striatum is required for a clinical response and that more than approximately 80% occupancy causes extrapyramidal adverse effects. The occupancy range of dopamine D_2 receptors in the striatum by 8 to 24 mg/d of blonanserin was 59.1% to 81.3%. The dose range required for the range of optimal dopamine D_2 receptor occupancy in the

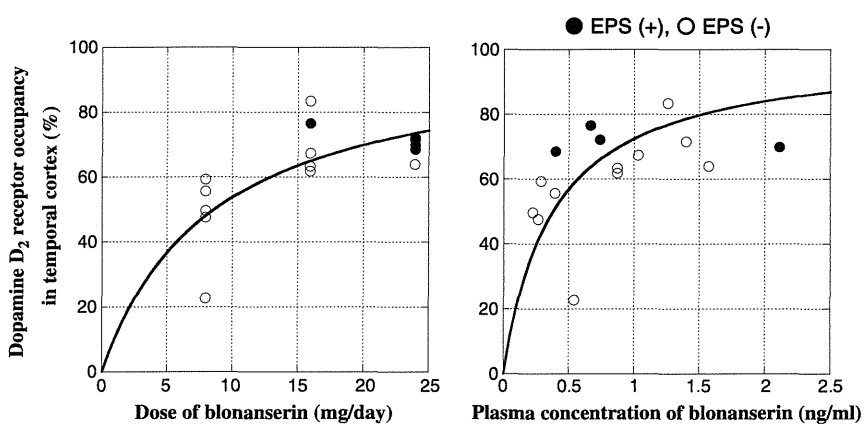

FIGURE 2. Relationship between dopamine D_2 receptor occupancy in the temporal cortex and dose or plasma concentration of blonanserin. ED_{50} in the temporal cortex was 8.61 mg/d ($r = 0.71$) and EC_{50} was 0.38 ng/mL ($r = 0.13$) (ED_{50}, dose required to induce 50% occupancy; EC_{50}, plasma concentration required to induce 50% occupancy).

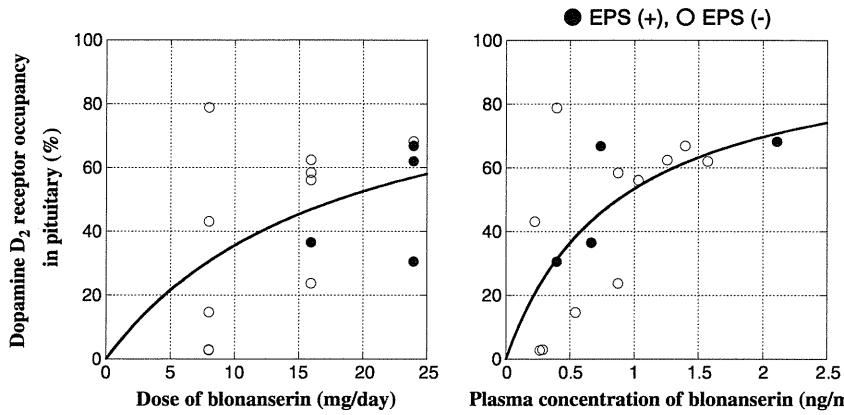

FIGURE 3. Relationship between dopamine D_2 receptor occupancy in the pituitary and dose or plasma concentration of blonanserin. ED_{50} in the pituitary was 18.06 mg/d ($r = 0.52$) and EC_{50} was 0.87 ng/mL ($r = 0.60$) (ED_{50}, dose required to induce 50% occupancy; EC_{50}, plasma concentration required to induce 50% occupancy).

striatum, calculated from ED_{50} and EC_{50}, was 12.9 to 22.1 mg/d and 0.44 to 0.76 ng/mL, respectively. The corresponding dose range to another therapeutic window of 60% to 78% D_2 occupancy suggested by a systematic review[13] was 8.3 to 19.6 mg/d. Thus, dopamine D_2 receptors of patients with schizophrenia might be almost optimally occupied when they are treated with the approved clinical daily dose range of 8 to 24 mg/d of blonanserin. However, more than 20 mg/d of blonanserin may have a higher incidence of EPS than a lower dose.

From another viewpoint, it should be noted that the dose-setting for blonanserin based on clinical trials in which a larger population of patients needed to be included showed good consistency with the optimal dose suggested by D_2 receptor occupancies investigated in a small number of patients. This suggested the validity and usefulness of dose-setting for antipsychotic drugs using PET.

Our results that patients taking 24 mg of blonanserin had an average dopamine D_2 occupancy in the striatum of approximately 80% and that 3 of 5 patients showed apparent EPS seem to be consistent with the hypothesis that more than approximately 80% occupancy causes extrapyramidal adverse effects. When examining striatal dopamine D_2 receptor occupancy in patients with apparent EPS individually, 3 patients had approximately 80%, but 1 patient had approximately 70%. Several factors such as sensitivity to antipsychotics, effect of previous medications, drug interaction, etc, might explain this result, but at this time it cannot be definitively stated why some patients with lower dopamine D_2 receptor occupancy in the striatum show EPS. Further study on the sensitivity to antipsychotics might one day answer this question.

Blonanserin has a high affinity for D_3 receptors as well as D_2 receptors [K_i value is 0.494 (0.137) nmol/L for D_3 receptors, and 0.142 (0.002) nmol/L for D_2 receptors],[5] and the D_2/D_3 affinity ratio was 0.287. This D_2/D_3 affinity ratio was higher than that of risperidone (0.157) and lower than those of quetiapine and ziprasidone (2.059 and 2.174, respectively).[34] Although the D_2/D_3 affinity ratio should not be neglected, in this study we could not distinguish dopamine D3 receptor binding from dopamine D_2 receptor binding by blonanserin, because both [^{11}C]raclopride and [^{11}C]FLB 457 also have affinity for dopamine D_3 receptors.

Interestingly, some PET studies reported that dopamine D_2 receptor occupancy in the extrastriatum was higher than in the striatum among atypical antipsychotics[35–37] and proposed the concept of "limbic selectivity" for the characteristics of atypical antipsychotics. We compared the striatal and extrastriatal dopamine D_2 receptors using different radioligands, [^{11}C]raclopride for the striatum and [^{11}C]FLB 457 for the extrastriatum, and we demonstrated that the average dopamine D_2 occupancy in the temporal cortex measured by [^{11}C]FLB 457 was 9.2% lower than that in the striatum measured by [^{11}C]raclopride. The dissociation constant K_d, indicating affinity for receptors in the living human brain, was quite different between [^{11}C]raclopride and [^{11}C]FLB 457. Although non-negligible specific binding in the cerebellum by [^{11}C]FLB 457 and differences in K_d value between 2 different radioligands cause systematic errors in occupancy,[22,38] the use of 2 tracers with different affinities, [^{11}C]raclopride and [^{11}C]FLB 457, must be superior compared with the use of 1 tracer to determine the occupancy in both the striatum and extrastriatum. Although direct comparisons of dopamine D_2 receptor occupancy between striatal and extrastriatal regions determined by different tracers may not be appropriate due to systematic errors in occupancy for [^{11}C]FLB 457 studies,[39] our findings suggested that the concept of "limbic selectivity" might not be applicable to blonanserin. However, the present study has demonstrated that dopamine D_2 receptor occupancy measured by [^{11}C]FLB 457 was lower than that by [^{11}C]raclopride, because the cerebellum has a somewhat higher affinity for [^{11}C]FLB 457 and non-negligible specific binding in the cerebellum might cause an underestimation of dopamine D_2 receptor occupancy by [^{11}C]FLB 457.[40,41] This possibility made it unclear whether the "limbic selectivity" of blonanserin should be excluded or not.

Another important finding was that the B/P ratio of blonanserin calculated from our data was 3.88 (5.53). Although it is difficult to compare it directly with the data for other antipsychotic drugs from our previous study,[8] blonanserin showed the highest B/P value in comparison to haloperidol [2.40 (2.40)], olanzapine [2.70 (1.84)], risperidone [1.61 (1.00)], and sulpiride [0.34 (0.42)] (data from Arakawa et al[8]). Other valuable findings from our study were that the average D_2 occupancy in the pituitary was less than 60% even at maximum dose, and ED_{50} in the pituitary was 2 times larger than in the temporal cortex. The prevalence of hyperprolactinemia by blonanserin was 20.0%. When we applied the same criteria of hyperprolactinemia in this study as in the previous study,[8] this prevalence was 20% for haloperidol, 14.3% for olanzapine, 57.1% for risperidone, and 100.0% for sulpiride. These findings might explain why the level of plasma concentration of prolactin did not elevate during the study, and support the hypothesis

that blonanserin shows relatively low risk of hyperprolactinemia as compared to other antipsychotics. These data were consistent with a previous study reporting that the blood prolactin level was lower with blonanserin as compared to risperidone.[6,7]

In conclusion, the results of dopamine D_2 receptor occupancy in the striatum by the approved clinical daily dose of blonanserin indicated that the optimal therapeutic dose of blonanserin for 70% to 80% D_2 occupancy was 12.9 to 22.1 mg/d. Blonanserin, which showed good permeability of BBB as expressed by a higher B/P ratio compared to other antipsychotics, poses a relatively low risk for hyperprolactinemia.

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Regular Article

Effects of menopause on brain structural changes in schizophrenia

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Aim: The aim of this study was to investigate the influences of menopause on brain morphological changes in schizophrenia using magnetic resonance imaging (MRI).

Methods: Forty female schizophrenia patients, 20 premenopausal and 20 postmenopausal, and 50 female controls underwent cerebral MRI. Optimized voxel-based morphometry was performed with Statistical Parametric Mapping version 5.

Results: Compared with controls, regional gray matter reductions in schizophrenia patients were observed in the insula, superior temporal gyrus, anterior cingulate, parahippocampal gyrus, and thalamus. Direct comparison between the patient groups showed that the gray matter of postmenopausal patients was significantly smaller when compared with premenopausal patients in the left middle frontal gyrus, and no region had significantly lower gray matter volume in premenopausal patients relative to postmenopausal patients. Significant negative correlation between gray matter volume and the interval after menopause was found in the right superior frontal gyrus in the postmenopause patient group.

Conclusion: Differential morphological alterations between postmenopausal and premenopausal schizophrenia patients were observed, suggesting that the female hormone plays a protective role against schizophrenia.

Key words: brain morphology, estrogen, female, menopause, schizophrenia

MANY STUDIES HAVE indicated the vulnerability to the pathological process of male schizophrenia patients relative to female patients. Female schizophrenia patients have lower antipsychotic dosage,[1] and a less malignant course of illness[2] than male patients. In addition, several studies have reported that schizophrenia has a later onset in female patients, with the first peak of onset in female patients occurring at age 25–29 years, in contrast to the peak of onset in male patients at age 20–24 years.[3] In addition, a second smaller peak of onset occurs in women after age 44 years, around perimenopause and menopause.[4,5] Based on these findings, it had been hypothesized that estrogen exerts a protective effect against the pathological process in schizophrenia.[5]

Studies regarding gender differences of brain morphology in schizophrenia had demonstrated more vulnerability in male patients than female patients. Magnetic resonance imaging (MRI) and postmortem studies have reported a tendency of greater abnormalities in male patients,[6] and male patients had larger lateral ventricle[6] and smaller medial temporal volume, that is, hippocampus and amygdala,[7] superior temporal gyrus,[7–9] and frontal[9] and temporal[10]

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lobe volumes than normal controls. Although these findings related to gender differences cannot be explained only by the degree of estrogen level, it could be hypothesized that the effect of estrogen partly reduces brain morphological changes in schizophrenia.

To our knowledge the relationship between estrogen and brain morphology in schizophrenia has not been reported in previous studies, although in postmenopausal female subjects without schizophrenia, several studies have reported the effect of estrogen therapy on brain atrophy.[11–13]

If female patients are under hormonal protection that can reduce the particular brain morphological changes of this disease, it could be suggested that premenopausal patients are under similar protection relative to postmenopausal patients. This would explain why only female patients have a second peak of onset in the paramenopausal phase.

In this study, we examined the influences of menopause on brain morphological changes of schizophrenia patients by classifying them into two subgroups, postmenopausal and premenopausal, using MRI. Although there is no previous study that investigated the effect of menopause on brain morphology of schizophrenia, studying gray matter (GM) volume by voxel-based morphometry (VBM)[14] has helped detect the localized effects of clinical characteristics, such as symptoms,[15] duration of illness,[16] and antipsychotic treatment,[17] and we considered VBM suitable for this investigation. We also examined whether the volumetric changes of postmenopausal patients were related to years elapsed after menopause.

METHODS

Subjects

Twenty female schizophrenia patients whose interval between last menstruation and the present MRI was at least 12 months were recruited and classified as the postmenopausal patient group. For the premenopausal patient group, 20 female schizophrenia patients matched to the postmenopausal patient group by age, and who had an interval between their last menstruation and the present MRI <12 months, were recruited.

The menstrual state of all patients was checked by interview. Twelve consecutive months of amenorrhea is the epidemiological definition of menopause according to Treloar.[18]

All patients fulfilling the diagnosis of schizophrenia as defined by DSM-IV criteria were recruited from the inpatient and outpatient facilities of Asai Hospital, Chiba, Japan. Diagnoses were made by the attending psychiatrists on the basis of a review of their charts and a conventional, semi-structured interview. In the 20 premenopausal patients, schizophrenia diagnosis was as follows: paranoid subtype, $n = 12$; disorganized subtype, $n = 1$; catatonic subtype, $n = 1$; undifferentiated subtype, $n = 1$; and residual subtype, $n = 5$. In the 20 postmenopausal patients the diagnosis was as follows: paranoid subtype, $n = 13$; disorganized subtype, $n = 0$; catatonic subtype, $n = 2$; undifferentiated subtype, $n = 2$; and residual subtype, $n = 3$, based on DSM-IV criteria.

The comparison group consisted of 50 healthy female volunteers matched with the patient groups by age, exclusion being based on a history of DSM-IV axis I or axis II disorder. Patients and comparison subjects were excluded if they had a history of head injury, neurological illness, or a diagnosis of substance abuse or dependence. Demographic and clinical characteristics are listed in Table 1.

All subjects were within the limited age range of 30–59 years. The mean age of premenopausal patients was 44.4 ± 8.0 years, postmenopausal patients, 46.8 ± 8.2 years; and normal controls, 45.0 ± 8.7 years. There were no significant differences in age between the groups according to diagnosis and menopause (analysis of variance, $P = 0.63$).

All patients were taking neuroleptic medication. Neuroleptic dosage in the premenopausal group was 959.5 ± 618.5 mg (chlorpromazine equivalent: atypical, $n = 7$; typical, $n = 5$; combined typical and atypical, $n = 8$). The dosage in the postmenopausal group was 746.8 ± 463.5 mg (chlorpromazine equivalent: atypical, $n = 10$; typical, $n = 4$; combined typical and atypical, $n = 6$). There were no significant differences in dosage or ratio of atypical, typical and combined therapy. Further, none of the patients was taking any hormonal therapy or other medication that could influence the level of sex hormones.

Patient symptoms were rated by Global Assessment of Functioning (GAF) and the Brief Psychiatric Rating Scale (BPRS).[19] BPRS total scores, as well as positive symptom (conceptual organization, unusual thought content, hallucinatory behavior) and negative symptom (emotional withdrawal, motor

Table 1. Subject characteristics

	Schizophrenia patients		Normal controls
	Premenopause	Postmenopause	
Variable (mean ± SD)	$n = 20$	$n = 20$	$n = 50$
Age (years)	44.4 ± 8.0	46.8 ± 8.2	45.0 ± 8.7
Period after menopause (years).	NA	5.0 ± 5.1	Unknown
Age at onset (years)	22.7 ± 10.6	24.9 ± 8.4	NA
Duration of illness (years)	21.0 ± 11.6	25.0 ± 13.0	NA
No. episodes	5 ± 3.9	7 ± 6.2	NA
GAF score	46 ± 11.0	42 ± 10.4	NA
Total BPRS score	49 ± 15.7	47 ± 18.2	NA
BPRS (P)	11.7 ± 3.9	10.7 ± 3.9	NA
BPRS (N)	9.8 ± 2.9	10.2 ± 4.0	NA
Education (years)	11.2 ± 1.9	11.6 ± 2.1	Unknown
Antipsychotics (*n*)			
Atypical	7	10	NA
Typical	5	4	NA
Combined typical and atypical	8	6	NA
Dosage (mg/day, chlorpromazine equivalent)	959.5 ± 618.5	746.8 ± 463.5	NA
Cumulative dosage (kg, chlorpromazine equivalent)	7.4 ± 7.3	6.3 ± 4.5	NA

BPRS, Brief Psychiatric Rating Scale; GAF, Global Assessment for Functioning; (N), negative symptoms; (P), positive symptoms.

retardation, blunted affect) subscales,[20,21] were used in the present analysis.

There were no significant differences between the patient subgroups in age at onset (two-tailed *t*-test, $P = 0.50$), duration of illness ($P = 0.41$), drug dosage ($P = 0.24$), GAF score ($P = 0.45$), total BPRS score ($P = 0.71$), BPRS positive symptom subscale ($P = 0.48$), negative symptom subscale ($P = 0.75$), or duration of education ($P = 0.46$). The average number of episodes, which represented the times they had been hospitalized because of worsening of their mental conditions, was five for the premenopausal patients and seven for the postmenopausal patients. This difference in number was not statistically significant ($P = 0.28$). Any characteristics except menopause were not significantly different between the patient subgroups.

This study was approved by the ethics committees of Nippon Medical School and Asai Hospital. After complete description of the study, all subjects gave written informed consent.

MRI acquisition

T1-weighted MRI was carried out on a 1.5-T GE Signa scanner (GE Medical Systems, Milwaukee, WI, USA). T1-weighted images were acquired in a coronal plane using an SPGR 3-D imaging sequence with the following parameters: TE = 9 ms, TR = 22 ms, flip angle = 30°, slice thickness = 1.5 mm, field of view = 25 cm, matrix = 256 × 192, voxel dimensions = 0.98 × 0.98 × 1.5 mm.

Image analysis

All images were organized for preprocessing and analyzed using SPM5 (Wellcome Department of Imaging Neuroscience, London, UK. http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) running in MATLAB 2008a (MathWorks, Natick, MA, USA). We used the optimized protocol as detailed by Good *et al.*[22]

We used the customized template of Asai Hospital. It was created from the MRI of 120 healthy subjects (60 male, 60 female) recruited from the local community. All the subjects were within the limited age range of 30–59 years, and were physically healthy at the time of scanning, and none had a history of current or past psychiatric illness, serious head injury, serious medical or neurological illness, or substance abuse.

Each structural MRI from all present schizophrenia and healthy control subjects was segmented into GM,

white matter (WM), and cerebral spinal fluid (CSF) compartments using the SPM priors. An automated brain extraction procedure that incorporated a segmentation step was used to remove non-brain tissue.[22] The extracted GM images were normalized to the created GM template. The normalization parameters obtained from this step were then applied to the original structural images in native space to reduce any contribution from non-brain voxels and afford optimal spatial normalization of GM. These normalized images were segmented into GM, WM, and CSF partitions.

Finally, all normalized, segmented GM images were smoothed with a 12-mm full width at half maximum (FWHM) isotropic Gaussian kernel.

Data analysis

The processed images were analyzed using SPM5. Volumes were compared according to two linear contrasts (more or less GM volume between subject groups).[23]

The resulting set of voxel values for each contrast constituted a statistical parametric map of the t-statistic (SPM-t). Comparisons were made between subject groups, organized by menopause and diagnosis. At first, the GM volume difference between female schizophrenia patients and female controls was investigated using SPM-t maps thresholded at $P < 0.05$ corrected by the family-wise error rate (FWE). Comparison was performed using an analysis of covariance (ANCOVA) model,[24] with age as covariate. We could not check the menopausal state of each of the healthy female subjects. Therefore, this first analysis was to demonstrate the overall tendency of brain morphology in female patients relative to healthy female subjects, without relation to menopause.

Then, the GM volume difference between postmenopausal patients and premenopausal patients was investigated using multivariate analysis of variance with age, antipsychotic medication, duration of illness, and BPRS negative symptoms as covariates (MANCOVA), at $P < 0.001$ uncorrected. The numerical value of the antipsychotic medication was the cumulative exposure calculated by the accumulation of the representative daily dosage of each year of their treatment histories. The representative dosage used was from the longest period of each year. The dosage was multiplied by the treated days of each year, and then accumulated through their treatment histories. The sum of the calculation for premenopausal patients was 7.4 ± 7.3 kg (chlorpromazine equivalent, mean ± SD), and the sum for postmenopausal patients was 6.3 ± 4.5 kg. There was no significant difference between the patient groups ($P = 0.57$).

Further, the relation of brain morphology of postmenopausal patients to years elapsed after menopause was examined using correlation analysis with age as covariate, at $P < 0.001$ uncorrected. Then we performed a region of interest analysis to investigate volume differences of the region significantly correlated with the interval after menopause in each postmenopausal patient, using the Marsbar toolbox.[25]

In the first analysis, we performed a comparison between 40 schizophrenia patients and 50 controls thresholded at $P < 0.05$ corrected. We used a different threshold at $P < 0.001$ uncorrected for the next two analyses, because these were done using a smaller sample size, that is, 20 postmenopausal patients and 20 premenopausal patients for the second analysis and 20 postmenopausal patients for the third analysis.

For visualization of group differences, the coordinates of significant voxels were converted from Montreal Neurological Institute space to Talairach and Tournoux coordinates[26] using a MATLAB conversion program written by Matthew Brett (MRC Cognition and Brain Sciences Unit, UK). Coordinates were then entered into Talairach Daemon[27] to localize results.

RESULTS

Brain regions with significant GM volume change in all analyses are shown in Tables 2–4 and Figs 1,2.

Female schizophrenia patients vs female controls

The regional GM of female schizophrenia patients was significantly smaller when compared with female control subjects ($P < 0.05$ corrected). Significant volume reductions were observed in the bilateral insula, left superior temporal gyrus, bilateral anterior cingulate, left parahippocampal gyrus, and left thalamus (Table 2; Fig. 1a).

No region had significantly lower GM volume in female controls relative to female schizophrenia patients.

Table 2. SPM5 regional GM reduction in patients relative to controls

		Schizophrenia patients < Controls				
			Coordinate			Cluster volume
Anatomical region	[Area]†	T	x	y	z	(mm^3)
Superior temporal gyrus	[41]Lt.	5.04	−46	−24	8	390
Insula	[13]Rt.	5.15	36	16	4	420
	[13]Lt.	6.02	−36	22	0	1880
Parahippocampal gyrus	[34]Lt.	4.98	−18	4	−18	130
Anterior cingulate	[25]Rt.	6.27	2	2	−6	2380
	[25]Lt.	6.39	−4	2	−4	2380
Thalamus	Lt.	5.2	−2	−8	0	2380

†Brodmann area. GM, gray matter; Lt., left hemispheric; Rt., right hemispheric.

Premenopausal patients vs postmenopausal patients

The regional GM of postmenopausal patients was significantly smaller when compared with premenopausal patients at the left middle frontal gyrus (Table 3; Fig. 1b; $P < 0.001$ uncorrected). In contrast, no region had significantly lower GM volume in premenopausal patients compared to postmenopausal patients.

Correlation between brain morphology of postmenopausal patients and interval after menopause

Correlation between brain morphology of postmenopausal patients and the interval after menopause is shown in Table 4 and Figs 1(c),2. The regional brain volume of the right superior frontal gyrus was significantly correlated with the interval after menopause: the longer the interval, the greater

Table 3. SPM5 regional GM reduction vs menopause in schizophrenia

		Postmenopausal patients < Premenopausal patients				
			Coordinate			Cluster volume
Anatomical region	[Area]†	T	x	y	z	(mm^3)
Middle frontal gyrus	[10]Lt.	4.24	−36	40	10	120

†Brodmann area. GM, gray matter; Lt., left hemispheric; Rt., right hemispheric.

Table 4. SPM5 negative correlation between GM volume and period of time after menopause

			Coordinate			Cluster volume
Anatomical region	[Area]†	T	x	y	z	(mm^3)
Superior frontal gyrus	[8]Rt.	4.83	20	38	38	340

†Brodmann area. GM, gray matter; Rt., right hemispheric.

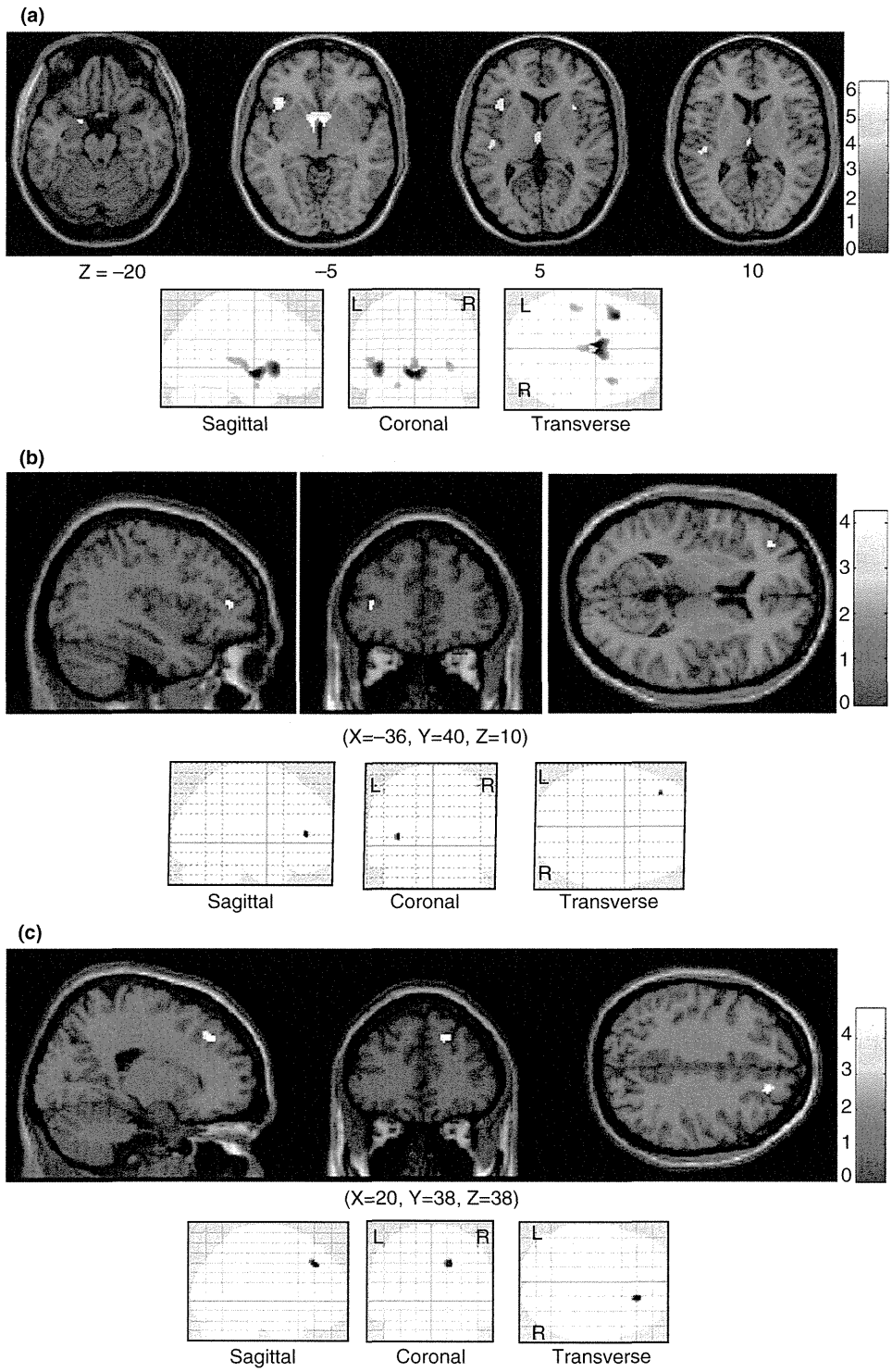

Figure 1. Voxel-based morphometry (VBM) analysis of T contrasts showing gray matter reductions in (a) female schizophrenia patients ($n = 40$) compared with female controls ($n = 50$; threshold $P < 0.05$, corrected); and (b) female postmenopausal patients ($n = 20$) compared with female premenopausal patients ($n = 20$; threshold $P < 0.001$, uncorrected). (c) VBM analysis of T contrasts showing the negative correlation between gray matter volume and the period of time after menopause in female postmenopausal patients ($n = 20$; threshold $P < 0.001$, uncorrected).

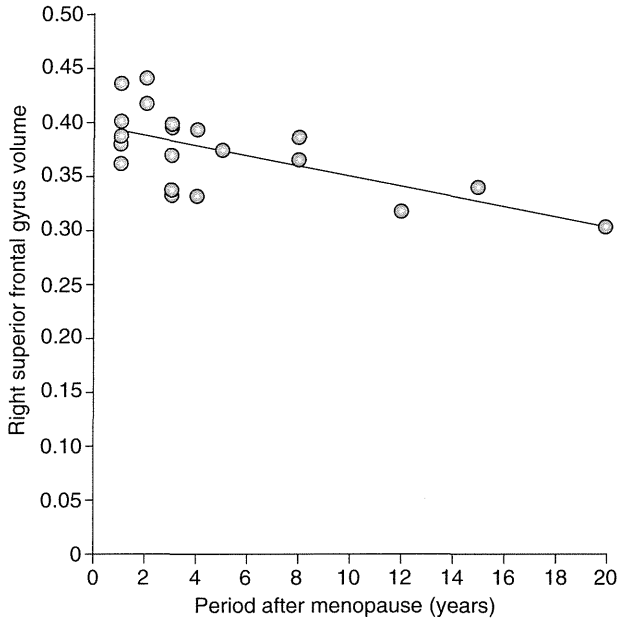

Figure 2. Correlation between gray matter volume of the right superior frontal gyrus and the period of time after menopause in female postmenopausal patients ($n = 20$).

the volume deficit in this region ($P < 0.001$ uncorrected). There was no significant correlation between longer postmenopausal interval and less volume deficit.

DISCUSSION

As a first step, we examined regional GM changes in female schizophrenia patients in relation to healthy female subjects by conducting VBM. The regions with remarkable GM volume reduction in patients were the insula, superior temporal gyrus, anterior cingulate, parahippocampal gyrus, and thalamus. The changes were predominantly found on the left side.

A meta-analysis reviewing 31 studies of VBM for GM volume in schizophrenia showed that patients had reduced GM density relative to control subjects in the bilateral insula, anterior cingulate, left postcentral gyrus, left parahippocampal gyrus, left middle frontal gyrus, and thalamus.[28] Another meta-analysis reviewed 15 studies of VBM for GM and WM volume in schizophrenia,[29] reporting that the areas significantly reduced in schizophrenia patients in >50% of the studies were the bilateral superior temporal gyrus, left medial frontal gyrus, left inferior frontal gyrus, and left parahippocampal gyrus.[29]

These two meta-analyses identified the morphology trait of schizophrenia using VBM. Generally, the present findings regarding patients versus controls are consistent with those of these meta-analyses in terms of locations and laterality.

We then examined the GM volume difference between postmenopausal patients and premenopausal patients. Direct comparison between the patient groups indicated morphological change in the left middle frontal gyrus. In addition, a significant correlation between brain morphology and interval after menopause was found in the right superior frontal gyrus. These regions are not identical, but both are located in the prefrontal area.

Volume reduction in the prefrontal area in schizophrenia patients has been observed in past studies, and morphological change in the prefrontal area has been indicated in relation to negative symptoms and cognitive impairment.[30] Additionally, a study regarding the relationship between illness duration and brain volume indicated that the right middle frontal cortex is particularly vulnerable to the long-term effect of schizophrenia.[16] According to these reports, the present results may indicate that postmenopausal patients are at a more advanced stage of progression.

A study on gender differences of brain morphology in schizophrenia reported that GM reductions in prefrontal areas were found predominantly in male patients.[9] It may be assumed that the duration of the low estrogen condition played a role in the morphological change in this area.

In the studies on menopause age of healthy subjects, the estimated median age at last menstruation period lies between 50 and 52 years.[31–33] A cross-sectional study concerning natural menopause of Japanese women reported that the median age of menopause was 50.54 years.[34]

The age of menopause of the postmenopausal patients in the present study, 41.8 ± 9.0 years, seemed considerably earlier than that of the healthy subjects. A major reason for this might be an antipsychotic medication-induced side-effect. Because of the very early age at menopause, the postmenopausal patient group in this study may not be a representative sample. This is a limitation of the study.

A study concerning the effect of antipsychotic-induced hyperprolactinemia in schizophrenia patients on antipsychotic medication showed that there was a significant inverse relationship between the prolactin levels in female patients and estradiol

levels, and when female patients with hyperprolactinemia were compared to those with a normal range of prolactin, only estradiol levels were significantly different between the two groups with regard to measured hormone levels (estradiol, testosterone, progesterone, luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone).[35]

According to that study, it could be assumed that the young patients in the postmenopausal group with probable hyperprolactinemia had low estrogen levels in the present study. The gradual decline of ovarian estrogen production begins in the years prior to menopause, although a dramatic decline in plasma estrogen level occurs at the final menstrual cycle.[36] Research concerning gender differences and age of onset in schizophrenia has shown a second peak of onset in female patients in the age range 45–54 years, and it was hypothesized that the vulnerability threshold for schizophrenia is raised in women until menopause due to the effect of estrogen.[37] Therefore, a gradual loss of the protective role of estrogen against brain morphological change may also begin prior to menopause.

In healthy subjects, according to studies investigating the effect of estrogen on brain morphology in postmenopausal women, some reported adverse effects of hormone therapy associated with greater atrophy in the hippocampal region[38] or in the putamen,[39] while several others demonstrated that estrogen therapy slows age-related GM loss in frontal cortices,[11,13] temporal, parietal, and occipital cortices,[11] cerebellum,[11,13] and the hippocampal region,[11,12] findings partially in agreement with the present ones. Therefore, it is suggested that the effect of menopause on brain structural changes in schizophrenia patients could be attributable to the loss of the protective effect of estrogen against the pathophysiology of schizophrenia.

A limitation of the present study is that blood concentrations of sex hormones were not measured. Therefore, the present results do not represent the effects of sex hormones but rather the effects of menopause. In addition, because neither hormone levels nor menstrual state were checked in the healthy controls, the main purpose the patients versus controls analysis was to demonstrate that the overall tendency of the present patients would reflect the previous studies concerning the brain morphology of schizophrenia.

In conclusion, volumetric comparisons showed differential morphological alterations due to female hormonal change in schizophrenia. Postmenopausal patients had more GM volume reduction than premenopausal patients at the left middle frontal gyrus. In addition, there was significant correlation between brain morphology and interval after menopause in the right superior frontal gyrus. These results support the hypothesis of the protective role of estrogen against schizophrenia.

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Quantification of Dopamine Transporter in Human Brain Using PET with ^{18}F-FE-PE2I

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^{18}F-(*E*)-*N*-(3-iodoprop-2*E*-enyl)-2β-carbofluoroethoxy-3β-(4-methylphenyl)nortropane (^{18}F-FE-PE2I) is a new PET radioligand with a high affinity and selectivity for the dopamine transporter (DAT). In nonhuman primates, ^{18}F-FE-PE2I showed faster kinetics and less production of radiometabolites that could potentially permeate the blood–brain barrier than did ^{11}C-PE2I. The aims of this study were to examine the quantification of DAT using ^{18}F-FE-PE2I and to assess the effect of radiometabolites of ^{18}F-FE-PE2I on the quantification in healthy humans. **Methods:** A 90-min dynamic PET scan was obtained for 10 healthy men after intravenous injection of ^{18}F-FE-PE2I. Kinetic compartment model analysis with a metabolite-corrected arterial input function was performed. The effect of radiometabolites on the quantification was evaluated by time-stability analyses. The simplified reference tissue model (SRTM) method with the cerebellum as a reference region was evaluated as a noninvasive method of quantification. **Results:** After the injection of ^{18}F-FE-PE2I, the whole-brain radioactivity showed a high peak (~3–5 standardized uptake value) and fast washout. The radioactive uptake of ^{18}F-FE-PE2I in the brain was according to the relative density of the DAT (striatum > midbrain > thalamus). The cerebellum showed the lowest uptake. Tissue time–activity curves were well described by the 2-tissue-compartment model (TCM), as compared with the 1-TCM, for all subjects in all regions. Time stability analysis showed stable estimation of total distribution volume with 60-min or longer scan durations, indicating the small effect of radiometabolites. Binding potentials in the striatum and midbrain were well estimated by the SRTM method, with modest intersubject variability. Although the SRTM method yielded a slight underestimation and overestimation in regions with high and low DAT densities, respectively, binding potentials by the SRTM method were well correlated to the estimates by the indirect kinetic method with 2-TCM. **Conclusion:** ^{18}F-FE-PE2I is a promising PET radioligand for quantifying DAT. The binding potentials could be reliably estimated in both the striatum and midbrain using both the indirect kinetic and SRTM methods with a scan duration of 60 min. Although radiometabolites of ^{18}F-FE-PE2I in plasma possibly introduced some effects on the radioactivity in the brain, the effects on estimated binding potential were likely to be small.

Key Words: ^{18}F-FE-PE2I; positron emission tomography; dopamine transporter; kinetic modeling; radiometabolite

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Dopamine transporter (DAT) plays a crucial role in the regulation of dopamine concentration in the synaptic cleft by dopamine reuptake. Changes in the density and function of DAT have been reported in various neuropsychiatric disorders, such as Parkinson disease (*1*), Huntington disease (*2*), attention-deficit/hyperactivity disorder (*3*), autism (*4*), and schizophrenia (*5*). Although DAT ligands for SPECT have been widely used in clinical practice, developing a useful radioligand for PET—which has higher resolution and better ability of quantification than SPECT—is the key to assessing its role in the pathophysiology of these diseases and to developing new therapeutic approaches for them.

Several radioligands for imaging DAT have been developed and used for PET. Among ^{11}C-labeled radioligands, ^{11}C-cocaine (*6*), ^{11}C-WIN35,428 (CFT) (*7*), ^{11}C-β-CIT (*8*), and ^{11}C-DL-threo-methylphenidate (*9*) have relatively low affinity for DAT or have slow kinetics in the high-DAT-density regions. ^{11}C-altropane has high affinity and selectivity for DAT (*10*), but the kinetics in the human brain have not been reported in detail to our knowledge. The ^{18}F-labeled radioligands that have been studied in humans so far include ^{18}F-CFT (^{18}F-WIN35,428) (*11*), *N*-3-fluoropropyl-2-β-carboxymethoxy-3-β-(4-iodophenyl)

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nortropane (^{18}F-FPCIT) (*12*), and 2β-carbomethoxy-3β-(4-chlorophenyl)-8-(2-fluoroethyl)nortropane (^{18}F-FECNT) (*13*). All of them have high affinity and selectivity for DAT, but the kinetics are relatively slow, and more than 90 min are needed to reach peak uptake in the striatum.

Recently, a new ligand, *N*-(3-iodoprop-2*E*-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)nortropane (PE2I), with a high affinity for DAT (inhibition constant, 17 nM) and good selectivity, was developed (*14,15*). In human PET studies, ^{11}C-PE2I showed a high specific-to-nonspecific ratio (*15–18*). However, 2 problems have been reported in quantifying DAT with ^{11}C-PE2I. First, because of the relatively slow kinetics of ^{11}C-PE2I in the striatum, reference tissue methods severely (~50%) underestimated DAT binding in this region, compared with those by the methods with arterial input function (*16,17*). Second, a radiometabolite of ^{11}C-PE2I has been found to cross the blood–brain barrier (BBB) in rats, thus potentially reducing the accuracy of the quantification of DAT (*19*).

A fluoroethyl analog of PE2I, ^{18}F-(*E*)-*N*-(3-iodoprop-2*E*-enyl)-2β-carbofluoroethoxy-3β-(4-methylphenyl)nortropane (^{18}F-FE-PE2I) (inhibition constant, 12 nM), has recently been developed and evaluated in nonhuman primates (*20*). In monkeys, ^{18}F-FE-PE2I was more favorable for the quantitative analysis of DAT, because it showed faster kinetics and less production of BBB-permeable radiometabolites than did ^{11}C-PE2I. The quantification of DAT with ^{18}F-FE-PE2I was less biased than that with ^{11}C-PE2I (*21*).

The aims of this study were to examine the method for quantification of DAT using ^{18}F-FE-PE2I and to assess the effect of radiometabolites of ^{18}F-FE-PE2I on the quantification in healthy humans.

MATERIALS AND METHODS

Subjects

Ten healthy men (mean age ± SD, 28.1 ± 6.9 y; age range, 20–39 y) participated in this study. All subjects were free of any somatic, neurologic, or psychiatric disorders. The study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiologic Sciences, Chiba, Japan. Written informed consent was obtained from all subjects before their inclusion in the study.

PET Procedure

^{18}F-FE-PE2I was synthesized from its acid precursor through a reaction with ^{18}F-2-bromo-1-fluoroethane in dimethylformamide and sodium hydroxide in *N,N*-dimethylformamide, as previously described (*22*).

A 90-min dynamic scan was obtained for each subject after a 1-min intravenous injection of ^{18}F-FE-PE2I using a PET scanner system (ECAT EXACT HR+; CTI-Siemens) The scan protocol consisted of 9 frames of 20 s, 5 frames of 1 min, 4 frames of 2 min, 12 frames of 4 min, and 5 frames of 6 min. The injected dose and specific activity were 183.0 ± 9.3 MBq and 146.1 ± 98.7 GBq/μmol at the time of injection, respectively. A head holder was used to minimize head movements. Scatter correction was performed. Attenuation correction was based on a transmission scan using a $^{68/68}$Ge/Ga source.

Arterial Blood Sampling and Metabolite Analysis

Arterial blood samples were taken manually 32 times after the injection of radioligand to obtain an arterial input function. Each blood sample was centrifuged to obtain plasma and blood cell fractions, and the concentration of radioactivity in whole blood and plasma was measured.

The fractions of the parent and its radiometabolites in plasma were determined by high-performance liquid chromatography (HPLC) from 10 blood samples for each subject. Each plasma sample had acetonitrile added and then was centrifuged. The supernatant of the centrifuged sample was subjected to radio-HPLC analysis (column, μBondapak C18 [Waters]). Acetonitrile (90%) (A) and phosphoric acid (0.01 M) (B) were used as mobile phases, with a flow rate of 6.0 mL/min. Gradient elution was used with the following gradient profile: 0–4.5 min, 25/75–70/30 A/B; 4.5–8.0 min, 70/30–25/75 A/B; and 8.0–10.0 min, 25/75–25/75 A/B. Linear interpolation was used to calculate the fractions of the parent and radiometabolites for the blood samples without metabolite analysis.

Image Analysis

T1-weighted MR images acquired with a 1.5-T MRI scanner (Gyroscan NT; Philips) (1-mm-slice axial images; repetition time, 21 ms; echo time, 9.2 ms; and flip angle, 30°) were coregistered to the corresponding PET images. Manually drawn volumes of interest were based on the anatomic information of MR images. Then, these volumes of interest were applied to the dynamic PET images to extract time–activity curves for the putamen, caudate, ventral midbrain (including the substantia nigra and ventral tegmental area), thalamus, and cerebellum. All image and kinetic analyses were performed using PMOD (version 3.0; PMOD Technologies).

Kinetic Analysis

Standard 1- and 2-tissue-compartment models (TCMs) (*18,23*) with an arterial input function (concentration of the parent in plasma) were used to estimate rate constants and total distribution volume (V_T) by an iterative nonlinear least-squares curve-fitting procedure without weighting. Linear interpolation was used to calculate the concentration of the parent in plasma at the time points of time–activity curves of tissue. The rate constants K_1 and k_2 represent the influx and efflux rates, respectively, for radioligand diffusion across the BBB. For 2-TCM, the rate constants k_3 and k_4 represent radioligand transfer between the compartments for nondisplaceable and specifically bound radioligand, respectively. V_T is equal to the ratio of the concentration of radioligand in tissue to that in plasma at equilibrium. Blood volume was fixed at 0.05 mL/mL (*24*). The binding potential (BP_{ND}) of ^{18}F-FE-PE2I was quantified by the indirect kinetic method. We used the cerebellum as the reference brain region because of its negligible DAT density as shown in a human autoradiographic study (*25*) and an in vivo displacement study in monkeys (*20*). BP_{ND} can be expressed as:

$$BP_{ND} = \left(V_{T(regions)}/V_{T(cerebellum)}\right) - 1,$$

where $V_{T(regions)}$ and $V_{T(cerebellum)}$ are V_T of target regions and the cerebellum, respectively.

Time–Stability Analysis

To investigate the effect of scan length on the estimation of V_T, we analyzed PET data while truncating scan length. The scan length from 90 to 40 min, with 10-min decrements, was analyzed to estimate V_T of varying scan lengths, using the parent concentration in plasma as the input function. For each region and duration,

V_T was expressed as the percentage of the V_T value obtained with a 90-min scan length.

Kinetic Analysis with Radiometabolite-Included Input Function

To assess the effect of lipophilic radiometabolites, we tested an alternative input function consisting of the concentration of the parent and the lipophilic radiometabolite, 4-hydroxymethyl analog of the parent (M1), to estimate rate constants and V_T.

Simplified Reference Tissue Model (SRTM)

^{18}F-FE-PE2I binding was also quantified by the SRTM method with the cerebellum as a reference region. Assuming that both target and reference regions have the same level of nondisplaceable binding, and the kinetics in the target and reference regions can be described by 1-TCM, BP_{ND} is obtained by solving the convolution equation using a nonlinear least-squares fitting procedure (*26*). In this method, the parameters are reduced to 3: R_1 (ratio of K_1 relative to the reference region), k_2, and BP_{ND}. In addition, to investigate the applicability of shorter study durations, BP_{ND} values estimated by the indirect kinetic method and by the SRTM method with 60-min scanning data were compared with those estimated with 90-min scanning data.

Statistical Analysis

The goodness of curve fitting of models with different levels of complexity was compared using the Akaike information criterion (AIC) (*27*) and F test. In a model with better fitting, AIC shows lower values. A P value of less than 0.05 was considered significant for the F test. The SE of kinetic parameters was given by the diagonal of the covariate matrix (*28*). Divided by the estimate of the parameter itself, SE was expressed as a percentage (SE/[estimates of the parameter]) and used to assess parameter identifiability. A smaller percentage indicates better identifiability. Pearson r and linear regression analyses were used to assess correlations between BP_{ND} values estimated with the different approaches. A paired t test was applied to assess the difference in BP_{ND} values between the indirect kinetic and SRTM methods.

RESULTS

Brain Uptake

After the injection of ^{18}F-FE-PE2I, the radioactivity was distributed throughout the brain, with a high peak (~3–5 SUV) and fast washout (Figs. 1 and 2A). The peak uptake occurred within 10 min in all regions. The rank order of radioactivity from approximately 15 min to the end of the scan was as follows: putamen and caudate >> midbrain > thalamus > cerebellum. The uptake in the midbrain was visible as 2 distinct regions. Specific binding, which is the difference in radioactivity between target regions and the cerebellum, reached peak levels within approximately 30 min after injection in all target regions (Fig. 2B). The ratio of radioactivity in the striatum to that in the cerebellum reached a peak level (~7.0) approximately 60 min after the injection and remained at almost the same level thereafter (Fig. 2C).

Plasma Analysis

Reversed-phase HPLC analysis of plasma resulted in the separation of the parent and 2 major radiolabeled components (Fig. 3A). The peak with longest retention time corresponded to the parent, representing approximately 14% of plasma radioactivity at 30 min after injection (Fig. 3B). Of the 2 major radiolabeled components, one (M1) was retained longer, representing approximately 20% of plasma radioactivity at 30 min after injection. The M1-to-parent ratio was stable (~1.3–1.4) at 20 min after injection. Retention of the other one (M2) was shorter and consisted of 2 peaks, which were not sufficiently separated from each other, representing approximately 70% of the plasma radioactivity at 30 min after injection. The concentration of the parent showed a quick peak and fast washout in plasma (Fig. 3C).

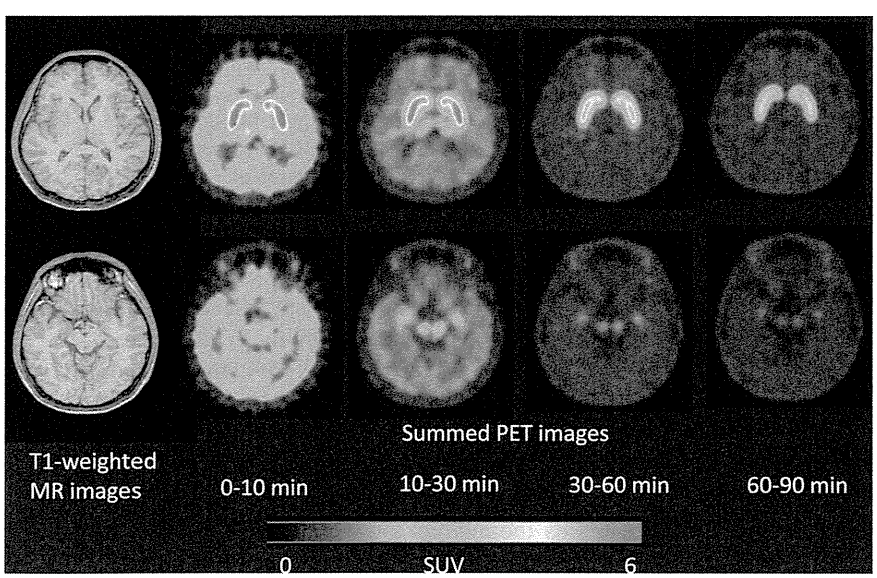

FIGURE 1. Representative dynamic PET images of healthy subject injected with ^{18}F-FE-PE2I. PET images were created at level of striatum (top) and midbrain (bottom). SUV = standardized uptake value.