Psychopharmacology (2021) 238:1343–1350
https://doi.org/10.1007/s00213-020-05698-3

ORIGINAL INVESTIGATION

Evaluation of dopamine D_3 receptor occupancy by blonanserin using [^{11}C]-(+)-PHNO in schizophrenia patients

Takeshi Sakayori[1] · Amane Tateno[1] · Ryosuke Arakawa[1] · Woo-chan Kim[1] · Yoshiro Okubo[1]

Received: 23 April 2020 / Accepted: 30 October 2020 / Published online: 12 November 2020

Abstract

Rationale Unlike other antipsychotics, our previous positron emission tomography (PET) study demonstrated that a single dose of blonanserin occupied dopamine D_3 as well as dopamine D_2 receptors in healthy subjects. However, there has been no study concerning the continued use of blonanserin.

Objectives We examined D_2 and D_3 receptor occupancies in patients with schizophrenia who had been treated with blonanserin.

Methods Thirteen patients with schizophrenia participated. PET examinations were performed on patients treated with clinical dosage of blonanserin or olanzapine alone. A crossover design was used in which seven patients switched drugs after the first scan, and PET examinations were conducted again. D_2 and D_3 receptor occupancies were evaluated by [^{11}C]-(+)-PHNO. We used nondisplaceable binding potential (BP_{ND}) of 6 healthy subjects which we previously reported as baseline. To consider the effect of upregulation of D_3 receptor by continued use of antipsychotics, D_3 receptor occupancy by blonanserin in seven subjects who completed 2 PET scans were re-analyzed by using BP_{ND} of olanzapine condition as baseline.

Results Average occupancy by olanzapine (10.8 ± 6.0 mg/day) was as follows: caudate 32.8 ± 18.3%, putamen 26.3 ± 18.2%, globus pallidus − 33.7 ± 34.9%, substantia nigra − 112.8 ± 90.7%. Average occupancy by blonanserin (12.8 ± 5.6 mg/day) was as follows: caudate 61.0 ± 8.3%, putamen 55.5 ± 9.5%, globus pallidus 48.9 ± 12.4%, substantia nigra 34.0 ± 20.6%. EC_{50} was 0.30 ng/mL for D_2 receptor for caudate and putamen (df = 19, $p < 0.0001$) and 0.70 ng/mL for D_3 receptor for globus pallidus and substantia nigra (df = 19, $p < 0.0001$). EC_{50} for D_3 receptor of blonanserin changed to 0.22 ng/mL (df = 13, $p = 0.0041$) when we used BP_{ND} of olanzapine condition as baseline.

Conclusions Our study confirmed that blonanserin occupied both D_2 and D_3 receptors in patients with schizophrenia.

Keywords D_3 receptor · Schizophrenia · Blonanserin · Positron emission tomography

Introduction

The dopamine D_2 receptor family contains 3 subtypes (D_2, D_3, and D_4), and they are known as the D_2-like receptor family. In terms of the treatment of schizophrenia, D_2 receptor has been thought to be strongly associated with the pathology of schizophrenia and be a major target for its treatment. Dopamine D_3 receptor has similarities to the other members of the D_2-like receptor family, but D_3 receptor has very high affinity for dopamine and modulates dopamine release as an autoreceptor (Gross and Drescher 2012). Dopamine D_3 receptors are predominantly located in the ventral striatum, thalamus, and hippocampus, which are important for psychotic symptoms and are thought to modulate normal dopaminergic function and cognition (Maramai et al. 2016). The results from PET studies with [^{11}C]-(+)-PHNO indicated that 100% of the signal in the substantia nigra (SN), 67% in the globus pallidus (GP), and 26% in the ventral striatum represent D_3 receptor sites (Searle et al. 2010). The distribution of D_3 receptor in the limbic areas indicated that D_3 receptor might regulate motivation and reward-related behavior (Leggio et al. 2013).

Selective D_3 receptor antagonists affect the firing of dopaminergic neurons in the ventral striatum in a manner similar to atypical antipsychotics, and they enhance dopamine and acetylcholine release in the prefrontal cortex (Millan et al. 2008). It has also been indicated that D_3 receptor antagonists can

This article belongs to a Special Issue on Imaging for CNS drug development and biomarkers.

✉ Yoshiro Okubo
okubo-y@nms.ac.jp

[1] Department of Neuropsychiatry, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113–8602, Japan

inhibit extrapyramidal symptoms and produce neither anhedonia nor metabolic adverse effects, mainly based on evidence from rodent studies (Richtand 2006; Young et al. 2012). D_3 receptor antagonists can improve a series of social and cognitive behaviors in rodents, including executive functions, which are particularly impaired in patients with schizophrenia, while D_2 antagonists do not have this effect (Gross et al. 2013). Since dopaminergic hypofunction in the prefrontal cortex has been implicated in the pathogenesis of negative symptoms (Davis et al. 1991) and cognitive dysfunctions of schizophrenia (Sawaguchi 2000), these findings led to the theoretical treatment model of D_3 receptor antagonism being a valuable approach for the treatment of schizophrenia (Maramai et al. 2016). Thus, it seems worth verifying whether D_3 receptor antagonism can improve the negative symptoms and cognitive deficits of schizophrenia.

Many antipsychotics have been reported with Ki values for D_2 receptors not differing much from those for D_3 receptors (McCormick et al. 2013), but occupancy of D_3 receptors is moderately less than that of D_2 receptors. It has been reported by a positron emission tomography (PET) study with [^{11}C]-(+)-PHNO that several antipsychotics (i.e., clozapine, risperidone, olanzapine) did not decrease, or even increased the in vivo nondisplaceable binding potential (BP_{ND}) of D_3 receptors in human brain (Graff-Guerrero et al. 2009). These findings suggested that these antipsychotics hardly occupied D_3 receptors in a clinical setting. [^{11}C]-(+)-PHNO gives a mixed $D_{2/3}$ signal composed of differing D_2 and D_3 proportions, and therefore previous studies measured BP_{ND} of D_3 receptors in D_3-receptor rich-regions. Another study also reported that chronically administered antipsychotics (i.e., clozapine, olanzapine, haloperidol) showed lower selectivity for D_3 compared with D_2 receptors ex vivo than in vitro in rat brain (McCormick et al. 2010).

Blonanserin is a second-generation antipsychotic drug developed in Japan, and it is currently being used as a therapeutic agent for schizophrenia in Japan, South Korea, and China. Comparative studies with other antipsychotic drugs have also been carried out, suggesting the possibility of this drug contributing to the improvement of cognitive impairments and negative symptoms of mental disorders (Murasaki 2016; Kishi et al. 2019). Blonanserin reportedly occupied a D_3-rich region (i.e., cerebellum lobes 9–10) similarly to a D_2-rich region (i.e., striatum) in rat brain, while risperidone, olanzapine, and aripiprazole did not (Baba et al. 2015). We recently examined the occupancy of D_2 and D_3 receptors by blonanserin in healthy subjects (Tateno et al. 2018). Using [^{11}C]-(+)-PHNO and PET, we demonstrated that a single dose of 12 mg of blonanserin occupied D_3 receptors to the same degree as D_2 receptors (i.e., EC_{50} for the D_2-rich region was 0.39 ng/mL and for the D_3-rich region was 0.40 ng/mL) (Tateno et al. 2018). This finding led us to suggest the possibility that some of the pharmacological effect of blonanserin in schizophrenia patients might be mediated via D_3 receptor antagonism. However, the result from the single-dose administration of blonanserin in healthy subjects may not reflect actual clinical practices as patients obtained antipsychotic effects by its continuous administration. Therefore, it is important to confirm how the continued use of blonanserin occupied D_3 receptor of patients with schizophrenia in a clinical setting.

We hypothesized that blonanserin would occupy D_3 receptor to the same degree as D_2 receptor in patients with schizophrenia, in a manner similar to healthy subjects. In the present study, we evaluated both D_2 and D_3 receptor occupancy by blonanserin in patients with schizophrenia and compared the results with those by olanzapine, which has been demonstrated as not occupying D_3 receptor (Mizrahi et al. 2011).

Methods

Subjects and study design

We selected a group of patients, aged 20 to 70 years, who met the criteria of Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV for schizophrenia. Inclusion criteria were as follows: (1) schizophrenia patients treated with blonanserin or olanzapine alone for 4 weeks or more, and those who did not change their dose for at least 2 weeks; (2) subjects who agreed to change from one drug to the other, (3) subjects who scored less than 120 on the Positive and Negative Syndrome Scale (PANSS15) at screening. Exclusion criteria were as follows: (1) subjects with past or current serious medical illness and/or organic brain diseases, (2) subjects with contraindication for the use of magnetic resonance imaging (MRI), (3) subjects with contraindication for blonanserin and olanzapine, (4) subjects treated with electroconvulsive therapy within 3 months before the screening, (5) subjects taking tandospirone at the time of screening, as there was a report that buspirone, which is in the same drug family, occupies D_3 receptor (Le Foll et al. 2016), and (6) subjects who were judged to be unsuitable for participation in this study. We allowed concomitant drugs such as benzodiazepines, antihypertensive drugs, and antiparkinsonian drugs that do not act on dopamine.

This study was designed as an open-label protocol. PET examination was performed on subjects who had been treated with blonanserin or olanzapine. A crossover design was then used in which patients switched drugs after the first scan, and PET examination was performed again after 2 weeks or longer. The doses of both drugs were within their clinical dose range. The mean dose of olanzapine was 10.8 ± 6.0 (range: 2.5–20) mg/day, and that of blonanserin was 12.8 ± 5.6 (8–24) mg/day. After complete explanation of the study, written informed consent was obtained from all participants. This study was approved by the institutional review board of Nippon Medical School Hospital, Japan.

Thirteen subjects participated in this study. The patients' characteristics are listed in Table 1. None of them worsened greatly after the medication change. Seven patients completed 2 PET scans, five of whom took olanzapine first and two were given blonanserin first. Six subjects were examined only by the 1st PET scan, 3 of whom participated in the PET scan with only blonanserin, and 3 with only olanzapine. Three of them felt uneasy about the drug change after the 1st PET scan and decided to continue with the initial drug, and the other 3 discontinued because of their clinical condition before the 2nd PET scan; one was diagnosed with diabetes , one complained of insomnia, and one was stopped due to extrapyramidal symptoms.

PET procedures

PET scans were performed with Eminence SET-3000GCT-X (Shimadzu Corp, Kyoto, Japan) to measure regional brain radioactivity. This scanner provides 99 sections with an axial field of view of 26.0 cm. Spatial resolution was 3.45 mm in-plane and 3.72 mm axially full-width at half-maximum. A head fixation device was used during the scans. A 15-min transmission scan was done to correct for attenuation using a ^{137}Cs source. Dynamic PET scan was performed for 90 min (1 min × 15, 5 min × 15) after i.v. bolus injection of [^{11}C]-(+)-PHNO. Injected radioactivity was 139.1 to 386.4 MBq (309.8 ± 79.7 (mean ± SD MBq) for olanzapine-condition; 351.2 ± 34.3 MBq for blonanserin-condition). The injected mass of [^{11}C]-(+)-PHNO was 0.5–2.5 μg (2.0 ± 0.7 μg for olanzapine-condition; 2.4 ± 0.3 μg for blonanserin-condition). Molar radioactivity was 55.3–141.0 GBq/μmol (86.0 ± 25.7 GBq/μmol for olanzapine-condition; 77.5 ± 21.9 GBq/μmol for blonanserin -condition) at the time of injection.

MRI procedures

MRI of the brain was acquired with 1.5 T MR imaging, Intera 1.5 T Achieva Nova (Philips Medical Systems, Best, Netherlands) as proton density image (echo time = 17 ms; repetition time = 6000 ms; field of view = 22 cm, 2-dimensional, 256 × 256; slice thickness = 2 mm; number of excitations = 2). These images were used for analysis of the PET scans.

Measurement of plasma concentrations of blonanserin and olanzapine

Venous blood samples were taken just before the PET scans, collected in tubes containing EDTA-2Na, and centrifuged at 3000 rpm for 10 min at 4 °C. Separated plasma samples were stored at − 80 °C until analysis. The plasma concentration of blonanserin was measured by validated method using high-performance liquid chromatography-tandem mass spectrometry with a target lower quantification limit of 0.001 ng/mL (Sekisui Medical Co., Ltd., Tokyo, Japan). The plasma concentration of olanzapine was measured by validated method

Table 1 Patient characteristics, dose, plasma concentration, and binding potential (BP_{ND}) of each ROI by olanzapine and blonanserin. *CAU*, caudate; *PUT*, putamen; *GP*, globus pallidus; *SN*, substantia nigra

ID	Gender	Age (years)	PANSS total	Olanzapine						Blonanserin					
				Dose (mg/day)	Plasma concentration (ng/mL)	Binding potential				Dose (mg/day)	Plasma concentration (ng/mL)	Binding potential			
						CAU	PUT	GP	SN			CAU	PUT	GP	SN
01	Male	62	76	*20*	67.3	0.77	1.09	2.62	2.38	*24*	0.851	0.42	0.62	0.76	0.44
02	Male	66	76	*10*	31.7	1.07	1.41	2.93	2.07	*N/A*	N/A	N/A	N/A	N/A	N/A
03	Female	69	53	*5*	21.9	0.93	1.17	2.35	1.82	*8*	0.414	0.58	0.77	1.11	0.60
04	Female	57	67	*20*	45.6	0.76	1.04	2.82	1.56	*N/A*	N/A	N/A	N/A	N/A	N/A
05	Male	46	62	*10*	29.4	0.98	1.30	3.34	4.31	*16*	0.518	0.71	0.92	1.24	0.86
06	Female	37	63	*5*	18.4	1.35	1.70	4.34	2.45	*8*	0.096	0.77	1.03	1.57	0.86
07	Female	46	72	*10*	50.7	1.30	1.72	3.83	3.43	*16*	0.856	0.62	0.90	0.86	0.56
08	Male	42	64	*N/A*	N/A	N/A	N/A	N/A	N/A	*8*	0.234	0.68	0.91	1.11	0.94
09	Male	55	53	*2.5*	13	1.02	1.31	2.38	1.73	*8*	1.08	0.69	0.88	1.27	0.80
10	Female	50	62	*15*	0.0161	1.52	1.84	2.33	0.98	*N/A*	N/A	N/A	N/A	N/A	N/A
11	Male	24	77	*10*	67.2	0.66	0.82	2.39	1.92	*16*	0.848	0.45	0.50	0.85	0.37
12	Female	47	84	*N/A*	N/A	N/A	N/A	N/A	N/A	*16*	0.421	0.42	0.64	0.89	0.59
13	Male	24	64	*N/A*	N/A	N/A	N/A	N/A	N/A	*8*	0.241	0.64	0.94	1.40	1.02
Average		48.1	67.2	10.8	34.5	1.04	1.34	2.93	2.27	12.8	0.56	0.60	0.81	1.11	0.70
SD		14.2	9.4	6.0	22.7	0.28	0.33	0.70	0.96	5.6	0.33	0.13	0.17	0.27	0.22

using high-performance liquid chromatography-tandem mass spectrometry with a target lower quantification limit of 0.0001 ng/mL (Sumika Chemical Analysis Service Co., Ltd., Osaka, Japan).

PET data analysis

MR images were co-registered to summated PET images with the mutual information algorithm using PMOD (version 3.4; PMOD Technologies Ltd., Zurich, Switzerland). Regions of interest (ROIs) were defined for the caudate, putamen, globus pallidus, substantia nigra, and cerebellum in accordance with Tziortzi's study (Tziortzi et al. 2011). We defined the caudate and putamen as D_2-rich regions and the substantia nigra and globus pallidus as D_3-rich regions, based on Searle's study with [^{11}C]-(+)-PHNO (Searle et al. 2010). ROIs were drawn manually on overlaid summated PET and co-registered MR images of each subject. By matching the targeted frame to the average of the first 10 frames (i.e., 0–10 min), motion corrections were conducted in all subjects.

Quantitative estimate of binding of [^{11}C]-(+)-PHNO was performed using a simplified reference tissue model (Lammertsma and Hume 1996), with the cerebellar cortex as reference region. We avoided cerebellum midline-structures because of measurable specific [^{11}C]-(+)-PHNO binding. This model has been validated to reliably estimate BP_{ND}, which compares the concentration of radioligand in the receptor-rich region with the receptor-free region (Innis et al. 2007) for [^{11}C]-(+)-PHNO (Ginovart et al. 2007).

Receptor occupancy by drugs was calculated by the following equation:

$$\text{Occupancy}\ (\%) = \left(BP_{NDbase} - BP_{NDdrug}\right)/BP_{NDbase} \times 100$$

BP_{NDdrug} is the BP_{ND} of schizophrenia patients treated with blonanserin or olanzapine. The BP_{ND} value of 6 healthy male volunteers (HVs) (age range 27–46 years; mean ± SD, 35.7 ± 7.6), which we reported in a previous study (Tateno et al. 2018), was used as baseline (BP_{NDbase}) (Table 2). Average BP_{ND} in the healthy volunteers under drug-free condition was as follows: caudate (range 1.04–1.68; mean ± SD 1.53 ± 0.24), putamen (1.28–2.06; 1.82 ± 0.29), globus pallidus (1.56–2.68; 2.16 ± 0.40), and substantia nigra (0.96–1.42; 1.06 ± 0.17).

We used a 1-site binding model, the same as in a previous study (Graff-Guerrero et al. 2010). The relationship between plasma concentration and receptor occupancy was shown by the following equation:

$$\text{Occupancy}\ (\%) = E_{max} \times C/(EC_{50} + C) \times 100,$$

where C is the plasma concentration of drug, E_{max} is the maximum occupancy, and EC_{50} is the plasma concentration required to achieve 50% occupancy (Tateno et al. 2018; Graff-Guerrero et al. 2010). E_{max} was fixed at 1 and $EC_{50} > 0$, the same as in the previous occupancy studies (Tateno et al. 2018; Graff-Guerrero et al. 2010).

Table 2 BP_{ND} values for the healthy volunteers in each region (Tateno et al. 2018). *HV*, healthy volunteers; *CAU*, caudate; *PUT*, putamen; *GP*, globus pallidus; *SN*, substantia nigra

ID	Gender	Age (years)	Drug-free			
			Binding potential			
			CAU	PUT	GP	SN
HV-1	Male	42	1.58	1.71	2.04	1.03
HV-2	Male	46	1.68	1.93	1.98	0.98
HV-3	Male	29	1.59	1.86	2.22	0.96
HV-4	Male	32	1.67	2.04	2.68	1.42
HV-5	Male	38	1.64	2.06	2.50	0.99
HV-6	Male	27	1.04	1.28	1.56	1.01
Average		35.7	1.53	1.82	2.16	1.06
SD		7.6	0.24	0.29	0.40	0.17

Mizrahi et al. reported that continuous intake of atypical antipsychotic drugs upregulated D_3 receptors (Mizrahi et al. 2011). Upregulation of D_3 receptors in treated schizophrenia patients might increase BP_{ND}, which induces the underestimation of occupancy of antipsychotics when using HV as baseline. To accurately compare D_3 receptor occupancy with D_2 receptor occupancy by blonanserin in consideration of the effect of the upregulation of D_3 receptors, we also calculated the D_3 receptor occupancy of blonanserin using individual BP_{ND} of olanzapine as a baseline among 7 patients who were taking both blonanserin and olanzapine. The paired *t* test was used to statistically analyze the comparison between D_3 receptor occupancy of blonanserin by using BP_{ND} of olanzapine as baseline and that of healthy control as baseline.

Results

The BP_{ND} values of each of the ROIs by olanzapine and blonanserin are summarized in Table 1.

D_2 and D_3 receptor occupancies by olanzapine and blonanserin

We analyzed D_2 and D_3 receptor occupancy using BP_{ND} of HV as baseline. The average occupancy by olanzapine (average ± SD, 10.8 ± 6.0 mg/day) was as follows: caudate nucleus 32.8 ± 18.3%, putamen 26.3 ± 18.2%, globus pallidus − 33.7 ± 34.9%, substantia nigra − 112.8 ± 90.7%. The average level of occupancy by blonanserin (12.8 ± 5.6 mg/day) was as follows: caudate nucleus 61.0 ± 8.3%, putamen 55.5 ± 9.5%,

globus pallidus 48.9 ± 12.4%, substantia nigra 34.0 ± 20.6%. Correlations between the plasma concentration of blonanserin and receptor occupancy in D_2-rich and D_3-rich regions are shown in Fig. 1. EC_{50} of D_2 receptor was 0.30 ng/mL (df = 19, $p < 0.0001$, 95% CI [0.215–0.394]), while EC_{50} of D_3 receptor was 0.70 ng/mL (df = 19, $p < 0.0001$, 95% CI [0.478–0.919]).

D_3 receptor occupancy by blonanserin using individual BP_{ND} of olanzapine as baseline

We also calculated the D_3 receptor occupancy by blonanserin using individual BP_{ND} of olanzapine condition as baseline. The results are shown in Table 3. The occupancy of D_3 was higher than when using the baseline BP_{ND} in healthy volunteers (67.9 ± 11.8% versus 44.7 ± 16.6%) (df = 26, p = 0.0002). EC_{50} of D_3 receptor occupancy was 0.22 ng/mL (df = 13, p = 0.0041, 95% CI [0.095–0.341]), which was close to that of D_2 receptor occupancy (Fig. 2).

Discussion

In this study, we confirmed that blonanserin indeed occupied D_3 receptors in the globus pallidus and substantia nigra, although to a lesser degree than D_2 receptors in the caudate nucleus and putamen, in patients with schizophrenia. On the other hand, olanzapine occupied 30% of the evaluation sites of D_2 receptor, but hardly those of D_3 receptor. These findings were consistent with a previous animal study and an in vivo human study (Baba et al. 2015; Graff-Guerrero et al. 2009).

The occupancy of D_3 receptor by blonanserin was a little lower than in a previous study of healthy volunteers (Tateno et al. 2018), but it was similar when recalculated using individual BP_{ND} under olanzapine condition. First, upregulation of D_3 receptors in treated schizophrenia patients with antipsychotics has been thought to influence BP_{ND} and the occupancy of antipsychotics (Graff-Guerrero et al. 2009; Mizrahi et al. 2011), and it might decrease the apparent D_3 receptor occupancy. Regarding the upregulation by antipsychotics, it was earlier reported that occupancy of the globus pallidus by clozapine, olanzapine, and risperidon was − 70.7 ± 86.5% (Graff-Guerrero et al. 2009). Another study reported that occupancy of the globus pallidus by olanzapine and risperidone was − 50.28 ± 29.37% (Mizrahi et al. 2011). In the current study, we also used BP_{ND} of D_3 receptors of patients under olanzapine treatment as a baseline for the calculation of D_3 receptor occupancy to reduce the influence of upregulation. Although it was expected to show a similar value to those of previous studies, our result was that the D_3 receptor occupancy by blonanserin (34.0 to 48.8%) was slightly lower than the D_2 receptor occupancy (55.5 to 61.0%) using HV as baseline. This result seemed to be due to the influence of upregulation. Second, schizophrenia patients showed increased [^{11}C]-(+)-PHNO binding compared to healthy subjects even if they were untreated (Weidenauer et al. 2020). For these reasons, individual baseline values would be more desirable. EC_{50} of D_3 receptor by blonanserin changed from 0.70 to 0.22 ng/mL when switching baseline BP_{ND} from the average of healthy volunteers to the individual patient's value with olanzapine in the 7 patients who had completed the 2 PET scans. This study assumes that the degree of upregulation was similar with olanzapine and blonanserin. This value was lower than EC_{50} of D_2 receptor (0.40 ng/mL) for blonanserin in the same 7 subjects by using HV as baseline. Thus, our results confirmed that blonanserin occupied D_3 receptor as well as D_2 receptor in patients with schizophrenia. Blonanserin might be an important target for further studies regarding the therapeutic efficacy of D_3 receptor blockade by antipsychotic drugs.

In this study, the degree of D_3 receptor occupancy in the substantia nigra by olanzapine in 8 patients with

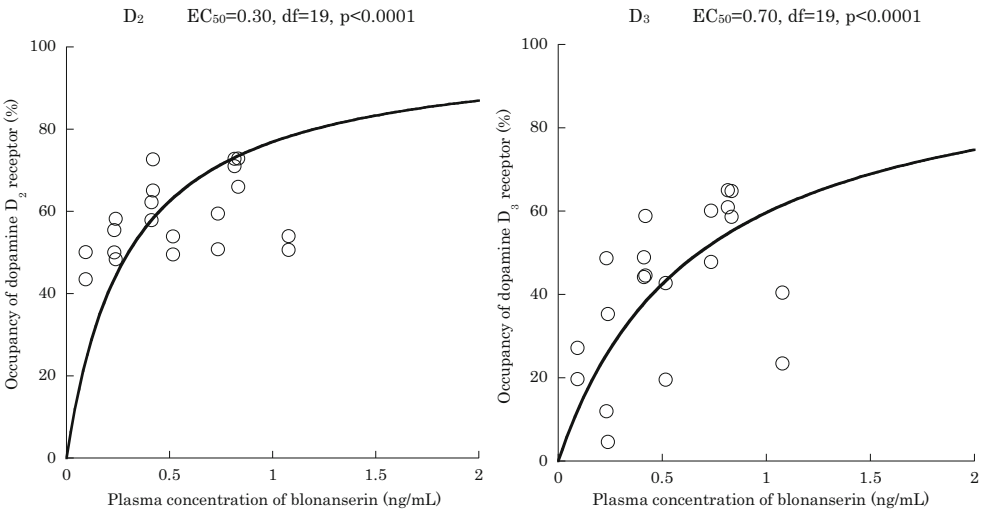

Fig. 1 Correlation diagram of blonanserin plasma concentration and dopamine D_2 (caudate nucleus and putamen) and D_3 (globus pallidus and substantia nigra) receptor occupancy (N = 10). Baseline BP_{ND} was the average value of healthy volunteers

Table 3 Dopamine D_2 receptor and D_3 receptor occupancy by blonanserin in seven patients who completed 2 PET scans. Baseline BP_{ND} was the average value of healthy volunteers for D_2 receptor and the individual value at olanzapine condition for D_3 receptor. *CAU*, caudate; *PUT*, putamen; *GP*, globus pallidus; *SN*, substantia nigra

ID	Occupancy of D_2-rich region (%)		Occupancy of D_3-rich region (%)	
	Baseline BP_{ND}: average value of healthy volunteers		Baseline BP_{ND}: individual value at olanzapine condition	
	CAU	PUT	GP	SN
01	72.8	65.9	70.9	81.4
03	62.1	57.8	52.9	67.2
05	53.8	49.5	62.9	80.1
06	50.0	43.4	63.8	65.1
07	59.4	50.7	77.5	83.8
09	53.9	50.6	46.7	53.7
11	70.9	72.7	64.5	80.6
Average	60.4	55.8	62.7	73.1
SD	8.8	10.3	10.4	11.3

schizophrenia was − 124.1 ± 87.6%. We thought that the negative occupancy might reflect upregulation. Previous studies did not measure the occupancy of the substantia nigra (Graff-Guerrero et al. 2009) or reported the combination of olanzapine (only one subject was included) and risperidone (Mizrahi et al. 2011). To our knowledge, this is the first report regarding the upregulation of dopamine D_3 receptor by olanzapine; however, the evaluation of a large number of subjects and/or using same subjects both before and after its administration will be needed for clarification.

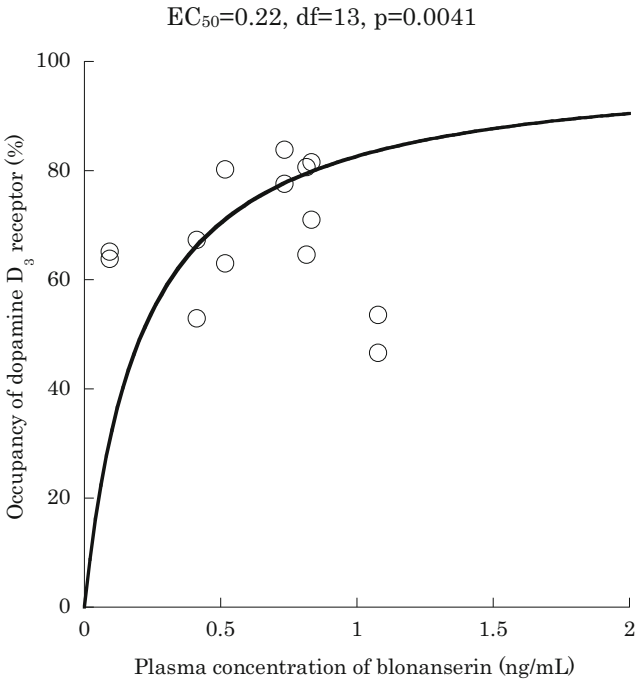

Fig. 2 Correlation diagram of blonanserin plasma concentration and D_3 (globus pallidus and substantia nigra) receptor occupancy (N = 7). Baseline BP_{ND} was the individual value under olanzapine condition

We should acknowledge several limitations to this study. First, our sample size was small. Furthermore, 6 of the 13 subjects underwent only one PET scan. Therefore, additional studies including larger numbers of subjects and longitudinal designs are essential for the generalization of our findings. Second, we used a younger-aged control group compared to the patients. BP_{ND} of D_2 receptor was negatively correlated with age in the caudate, while that of D_3 receptor was not correlated with age in the globus pallidus and substantia nigra (Nakajima et al. 2015). Our results of D_2 receptor using younger-aged controls for baseline might be influenced by an age effect if controls would be older. Third, we used all-male HVs, whereas 46% of the patients were female. In this regard, a previous study indicated that D_3 receptor differed between male and female rhesus monkeys (Martelle et al. 2014). Fourth, it is uncertain whether the degree of upregulation was similar or not between olanzapine and blonanserin. This study assumes that the degrees were comparable, although we could not estimate D_3 upregulation exactly as there was no drug-free baseline condition. Fifth, 7 of the 13 participants in this study were smokers while all HVs were non-smokers. We could not rule out the effects of smoking, as it has been shown to have an effect on the dopamine system (Le Foll et al. 2014),

In conclusion, our study confirmed that continuous usage of blonanserin occupied dopamine D_3 receptors to the same degree as D_2 receptors in the brains of schizophrenia patients. By more discussions on the therapeutic effects of blonanserin, which is now known to clearly possess in vivo D_3 receptor antagonism, we may be able to consider the relevance of anti-dopamine D_3 receptor acivities as well as the therapeutic effects on cognitive impairments and negative symptoms of mental disorders.

Acknowledgments We are grateful to Dr. Alan A. Wilson for advice on the synthesis of [^{11}C]-(+)-PHNO. We thank Mr. Koji Nagaya, Mr. Koji Kanaya, Ms. Megumi Hongo, and Mr. Minoru Sakurai for their assistance in performing the PET experiments and MRI scanning, and to Ms. Michiyo Tamura for her help as clinical research coordinator (Clinical Imaging Center for Healthcare, Nippon Medical School, Tokyo, Japan).

Funding This study was conducted under an investigator-led founded research agreement between Nippon Medical School Hospital and Sumitomo Dainippon Pharma Co., Ltd. (Osaka, Japan). Based on that agreement, the founder had the right to collect the information on serious TEAEs due to the study drug but had no roles in the study's design, analysis or drafting.

Compliance with ethical standards

After complete explanation of the study, written informed consent was obtained from all participants. This study was approved by the institutional review board of Nippon Medical School Hospital, Japan.

Conflict of interest Author Y.O. has received grants or speaker's honoraria from Sumitomo Dainippon Pharma, GlaxoSmithKline, Janssen Pharmaceutical, Otsuka, Pfizer, Eli Lilly, Astellas, Yoshitomi, and Meiji within the past 3 years. The remaining authors declare no interests.

References

Baba S, Enomoto T, Horisawa T, Hashimoto T, Ono M (2015) Blonanserin extensively occupies rat dopamine D_3 receptors at antipsychotic dose range. J Pharmacol Sci 127:326–331

Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. Am J Psychiatry 148: 1474–1486

Ginovart N, Willeit M, Rusjan P, Graff A, Bloomfield PM, Houle S, Kapur S, Wilson AA (2007) Positron emission tomography quantification of [^{11}C]-(+)-PHNO binding in the human brain. J Cereb Blood Flow Metab 27:857–871

Graff-Guerrero A, Mamo D, Shammi CM, Mizrahi R, Marcon H, Barsoum P, Rusjan P, Houle S, Wilson AA, Kapur S (2009) The effect of antipsychotics on the high-affinity state of D_2 and D_3 receptors; a positron emission tomography study with [^{11}C]-(+)-PHNO. Arch Gen Psychiatry 66:606–615

Graff-Guerrero A, Redden L, Abi-Saab W, Katz DA, Houle S, Barsoum P, Bhathena A, Palaparthy R, Saltarelli MD, Kapur S (2010) Blockade of [^{11}C]-(+)-PHNO binding in human subjects by the dopamine D_3 receptor antagonist ABT-925. Int J Neuropsychopharmacol 13:273–287

Gross G, Drescher K (2012) The role of dopamine D_3 receptors in antipsychotic activity and cognitive functions. Handb Exp Pharmacol 213:167–210

Gross G, Wicke K, Drescher KU (2013) Dopamine D_3 receptor antagonism—still a therapeutic option for the treatment of schizophrenia. Naunyn Schmiedeberg's Arch Pharmacol 386:155–166

Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE (2007) Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 27:1533–1539

Kishi T, Matsui Y, Matsuda Y, Katsuki A, Hori H, Yanagimoto H, Sanada K, Morita K, Yoshimura R, Shoji Y, Hagi K, Iwata N (2019) Efficacy, tolerability, and safety of blonanserin in schizophrenia: an updated and extended systematic review and meta-analysis of randomized controlled trials. Pharmacopsychiatry 52: 52–62

Lammertsma AA, Hume SP (1996) Simplified reference tissue model for PET receptor studies. Neuroimage 4:153–158

Le Foll B, Pushparaj A, Pryslawsky Y, Forget B, Vemuri K, Makriyannis A, Trigo JM (2014) Translational strategies for therapeutic development in nicotine addiction: rethinking the conventional bench to bedside approach. Prog Neuro-Psychopharmacol Biol Psychiatry 52:86–93

Le Foll B, Payer D, Di Ciano P, Guranda M, Nakajima S, Tong J, Mansouri E, Wilson AA, Houle S, Meyer JH, Graff-Guerrero A, Boileau I (2016) Occupancy of dopamine D3 and D2 receptors by buspirone: A [^{11}C]-(+)-PHNO PET study in humans. Neuropsychopharmacology 41:529–537

Leggio GM, Salomone S, Bucolo C, Plantania C, Micale V, Caraci F, Drago F (2013) Dopamine D_3 receptor as a new pharmacological target for the treatment of depression. Eur J Pharmacol 719:25–33

Maramai S, Gemma S, Brogi S, Campiani G, Butini S, Stark H, Brindisi M (2016) Dopamine D_3 receptor antagonists as potential therapeutics for the treatment of neurological disease. Front Neurosci 10:451 eCollection 2016

Martelle SE, Nader SH, Czoty PW, John WS, Duke AN, Garg PK, Garg S, Newman AH, Nader MA (2014) Further characterization of quinpirole-elicited yawning as a model of dopamine D3 receptor activation in male and female monkeys. J Pharmacol Exp Ther 350(2):205–211

McCormick PN, Kapur S, Graff-Guerrero A, Raymond R, Nobrega JN, Wilson AA (2010) The antipsychotics olanzapine, risperidone, clozapine, and haloperidol are D_2-selective ex vivo but not in vitro. Neuropsychopharmacology 35:1826–1835

McCormick PN, Wilson VS, Wilson AA, Remington GJ (2013) Acutely administered antipsychotic drugs are highly selective or dopamine D_2 over D_3 receptors. Pharmacol Res 70:66–71

Millan MJ, Loiseau F, Dekyne A, Gobert A, Flik G, Cremers TI, Rivet JM, Sicard D, Billiras R, Brocco M (2008) S33138 (N-[4-[2-(3aS, 9bR)-8-cyano-1,3a,4,9b-tetrahydro[1] benzopyrano[3,4-c]pyrrol-2(3H)-yl)-ethyl] phenyl-acetamnide), a preferential dopamine D_3 versus D_2 receptor antagonist and potential antipsychotic agent: III. Actions in models of therapeutic activity and induction of side effects. J Pharmacol Exp Ther 324:1212–1226

Mizrahi R, Agid O, Borlido C, Suridjan I, Rusjan P, Houle S, Remington G, Wilson AA, Kapur S (2011) Effects of antipsychotics on D_3 receptors: a clinical PET study in first episode antipsychotic naïve patients with schizophrenia using [^{11}C]-(+)-PHNO. Schizophr Res 131:63–68

Murasaki M (2016) The world's first dopamin serotonin antagonist? -the breakthrough of blonanserin- (Japanese). Rinsyoseishinyakuri 19: 213–244

Nakajima S, Caravaggio F, Boileau I, Chung JK, Plitman E, Gerretsen P, Wilson AA, Houle S, Mamo DC, Graff-Guerrero A (2015) Lack of age-dependent decrease in dopamine D_3 receptor availability: A [^{11}C]-(+)-PHNO and [^{11}C]-raclopride positron emission tomography study. J Cereb Blood Flow Metab 35:1812–1818

Richtand NM (2006) Behavioral sensitization, alternative splicing, and D3 dopamine receptor-mediated inhibitory function. Neuropsychopharmacology 31:2368–2375

Sawaguchi T (2000) The role of D1-dopamine receptors in working memory-guided movements mediated by frontal cortical areas. Parkinsonism Relat Disord 7:9–19

Searle G, Beaver JD, COmley RA, Bani M, Tziortzi A, Slifstein M, Mugnaini M, Graffante C, Wilson AA, Merlo-Pich E, Houle S, Gunn R, Rabiner EA, Laruelle M (2010) Imaging dopamine D_3 receptors in the human brain with positron emission tomography, [^{11}C]PHNO, and selective D_3 erceptor anagonist. Biol Psychiatry 68:392–399

Tateno A, Sakayori T, Kim WC, Honjo K, NakayamaH AR, Okubo Y (2018) Comparison of dopamine D_3 and D_2 receptor occupancies by a single dose of blonanserin in healthy subjects: a positron emission tomography study with [^{11}C]-(+)-PHNO. Int J Neuropsychopharmacol 21:522–527

Tziortzi AC, Searle GE, Tzimopoulou S, Salinas C, Beaver JD, Jenkinson M, Laruelle M, Rabiner EA, Gunn RN (2011) Imaging dopamine receptors in humans with [^{11}C]-(+)-PHNO: dessection of D_3 signal and anatomy. Neuroimage 54:264–277

Weidenauer A, Bauer M, Sauerzopf U, Bartova L, Nics L, Pfaff S, Philippe C, Berroterán-Infante N, Pichler V, Meyer BM, Rabl U, Sezen P, Cumming P, Stimpfl T, Sitte HH, Lanzenberger R, Mossaheb N, Zimprich A, Rusjan P, Dorffner G, Mitterhauser M, Hacker M, Pezawas L, Kasper S, Wadsak W, Praschak-Rieder N, Willeit M (2020) On the relationship of first-episode psychosis to the amphetamine-sensitized state: a dopamine D 2/3 receptor agonist radioligand study. Transl Psychiatry 10(1):2

Young JW, Amitai N, Geyer MA (2012) Behavioral animal models to assess pro-cognitive treatments for schizophrenia. Handb Exp Pharmacol 213:39–79