Striatal and Extrastriatal D_2/D_3-Receptor–Binding Properties of Ziprasidone

A Positron Emission Tomography Study With [^{18}F]Fallypride and [^{11}C]Raclopride (D_2/D_3-Receptor Occupancy of Ziprasidone)

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Abstract: To elucidate the "atypicality" of ziprasidone, its striatal and extrastriatal D_2/D_3-receptor binding was characterized in patients with schizophrenia under steady-state conditions. These data were compared with striatal receptor occupancy values after single-dose ziprasidone ingestion in healthy controls. [^{18}F]fallypride positron emission tomography (PET) recordings were obtained in 15 patients under steady-state ziprasidone treatment at varying time points after the last dose. Binding potentials were calculated for striatal and extrastriatal regions. D_2/D_3-receptor occupancies were expressed relative to binding potentials in 8 unmedicated patients. In a parallel [^{11}C]raclopride-PET study, striatal D_2/D_3-receptor occupancy was measured in healthy subjects after single oral doses of 40 mg ziprasidone or 7.5 mg haloperidol. Ziprasidone plasma concentrations correlated significantly with D_2/D_3-receptor occupancies in all volumes of interests. Occupancy in extrastriatal regions was approximately 10% higher than in striatal regions. Half maximal effective concentration values were consistently higher in striatal than in extrastriatal regions (temporal cortex: 39 ng/mL; putamen: 64 ng/mL), irrespective of the time between last dosing and scan. Single ziprasidone doses resulted in higher occupancies exceeding the 95% prediction limits of the occupancy versus plasma concentrations for chronic dosing. Ziprasidone shares moderate preferential extrastriatal D_2/D_3-receptor binding with some other atypicals. D_2/D_3-receptor occupancy is rapidly attuning to the daily course of ziprasidone plasma levels, suggesting relatively high intraday variations of D_2/D_3-receptor binding. The discrepancies between single-dose and steady-state results are important for the future design of dose-finding PET occupancy studies of novel antipsychotics. Single-dose studies may not be totally relied on for final dose selection.

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The second-generation antipsychotic (SGA), ziprasidone, has proven efficacy in the treatment of schizophrenia and mania.[1,2] Ziprasidone combines an antipsychotic efficacy similar to that of the prototypic first-generation antipsychotic (FGA), haloperidol, with the advantages of moderate extrapyramidal and minimal occurrence of sexual, vegetative, and metabolic side effects.[3,4] Ziprasidone is a D_2/D_3 dopamine and 5-HT_2 serotonin-receptor antagonist of relatively high affinity. In addition, it is a partial 5-HT_{1A}–receptor agonist and a serotonin and noradrenaline transporter reuptake inhibitor.[5,6] Previous positron emission tomography (PET) or single-photon emission computed tomography investigations revealed 60% to 75% striatal D_2/D_3-receptor occupancy in patients obtaining clinically effective doses/plasma concentrations.[7–9] However, the extent of ziprasidone binding to extrastriatal D_2/D_3 dopamine receptors has not yet been determined.

With the advent of high-affinity radiotracers belonging to the class of substituted benzamides, it became possible to quantify extrastriatal dopamine receptors in living human brain. One of the most widely used radiotracers for the quantification of extrastriatal D_2/D_3 receptors is [^{18}F]fallypride ([^{18}F]FP).[10,11] It displays similarly very high affinities for D_2 and D_3 receptors and negligible affinity for any other abundant neuroreceptor.[12] Although earlier PET studies with the moderate-affinity D_2/D_3 antagonist [^{11}C]raclopride ([^{11}C]RAC) demonstrated that the SGAs clozapine and

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quetiapine occupy striatal D_2/D_3 receptors to a significantly lesser extent than do other antipsychotics,[13–15] it was later consistently shown in PET studies with high-affinity ligands that these 2 compounds nonetheless occupy a significantly higher proportion of temporolimbic than striatal D_2/D_3 receptors.[16,17] We have demonstrated that the clinical antipsychotic efficacy of clozapine is likely more related to its binding in temporal cortex than to its striatal binding.[16] This "preferential" extrastriatal binding of SGAs, which was initially observed by Pilowsky et al,[18] has since been shown for several other SGAs, at least to a modest extent.[19,20] The potential reasons for some controversial aspects of this phenomenon[21] have been discussed previously.[22,23]

To predict the appropriate clinical ziprasidone dose, receptor occupancy was initially characterized in [^{11}C]RAC-PET studies of normal volunteers. Because a single 60-mg dose of ziprasidone occupied 85% of striatal D_2/D_3 receptors, it was predicted that "an effective antipsychotic dose will be between 20 and 40 mg."[7] Furthermore, a single low dose of ziprasidone evoked high occupancy (>70%) of cortical 5-HT_{2A} receptors.[24] However, the antipsychotic dose in clinical studies was in fact 120 mg/d or more; doses of 40 mg/d or lower were not superior to placebo.[25,26] It was subsequently determined that the D_2/D_3-receptor occupancy under steady-state conditions in subchronically treated patients was lower than after acute administration.[9] Thus, these results reveal an incongruity of the dopamine-receptor occupancy after single-dose and steady-state treatment with ziprasidone. Apparently, single-dose PET experiments of ziprasidone D_2/D_3-receptor occupancies predicted much lower daily doses than were later found to be clinically effective.

To further characterize ziprasidone's extrastriatal binding and temporal changes in its binding in relation to its serum concentration, we performed PET studies with [^{18}F]FP in patients with schizophrenia at varying time points after the last drug administration. Furthermore, to investigate the basis of the discrepant effects of single versus multiple dosing, we used [^{11}C]RAC-PET to measure the occupancy of striatal dopamine D_2/D_3 receptors by acute ziprasidone or haloperidol in normal subjects.

MATERIALS AND METHODS

Ziprasidone Occupancy Study Under Steady-State Treatment

The study was approved by the local ethics committee and the radiation safety authorities. A total of 23 inpatients with schizophrenia or schizoaffective disorder (according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria) were included after giving written informed consent. All subjects underwent physical and mental-state examinations. Patients with additional neurological or relevant somatic diseases were not included, nor were patients reporting illegal drug consumption in the preceding 6 months.

Patients

A subgroup of 15 patients (11 men, 4 women; 20–44 years; mean, 30 years [SD, 8 years]) was treated with stable doses of ziprasidone (80–200 mg/d; mean, 143 mg/d [SD, 35 mg/d]) for at least 1 week. No patient received comedication with antidopaminergic, anticholinergic, or antiserotoninergic properties. Four patients needed concomitant benzodiazepine or zolpidem treatment, 3 patients received serotonin reuptake inhibitors, and 1 patient was treated with valproic acid. The patients were assigned to 2 groups with different time intervals between the last ziprasidone ingestion and the PET scan (short interval, <10 hours, n = 7; long interval, >10 hours, n = 8). As it was one goal of the study to determine possible differences in the plasma concentration/receptor occupancy relationship, it was necessary to ensure that, in the short- and long-interval groups, the ziprasidone plasma levels during the PET scan were not significantly different. Therefore, patients with higher daily doses or trough plasma levels, respectively, were allocated to the long-duration group if clinically justifiable. The control group consisted of 5 male and 3 female patients (18–58 years; mean, 31 years [SD, 14 years]) who were drug- and medication-free for at least 6 months. The treated patients did not significantly differ from the unmedicated subjects in age (unpaired 2-tailed t test, −0.47 years; $P = 0.920$).

Radiochemistry and Data Acquisition

The radiosynthesis of [^{18}F]FP was a high-yield modification of the method for the synthesis of [^{18}F]desmethoxyfallypride, as described in detail previously.[27] Emission images were acquired on a Siemens ECAT EXACT 922/47 whole-body PET scanner (Siemens/CTI, Knoxvillle, Tenn) in 3D mode (field of view = 16.2 cm; 47 planes; full width at half maximum axial = 4.6 mm; in-plane = 6.0 mm). Data consisted of 39 time frames (3 × 20 seconds and 3 × 1, 3 × 2, 3 × 3, 21 × 5, 2 × 8, and 4 × 10 minutes), resulting in a total recording period of 180 minutes. Previous evaluation studies using protocols up to 240 minutes[11] found 180-minute scan durations sufficient for adequate binding potentials (BP_{ND}) calculation in striatal and extrastriatal regions. A 15-minute transmission scan using a ^{68}Ge source was carried out before each study for subsequent attenuation correction. Fallypride was injected intravenously as a bolus into a cubital vein over approximately 30 seconds (treatment group: 105–264 MBq; mean, 217 MBq [SD, 42 MBq]; control group: 176–349 MBq; mean, 233 MBq [SD, 6 MBq]). During the PET scan, venous blood samples were taken to measure the ziprasidone plasma concentrations at 0, 90, and 180 minutes after FP injection. Ziprasidone was determined in serum by high-performance liquid chromatography with column switching as described previously.[28]

Image and Data Analysis

Images were reconstructed with filtered backprojection using a Ramp filter and a Hanning filter (filter width = 4 mm). After motion correction, the whole dynamic scan was spatially normalized into Montreal Neurological Institute coordinates using the MEDx software (v3.43, Medical Numerics; Germantown, Md). A template of polygonal volumes of interest (VOIs) was used to calculate time-activity curves for cerebellum, caudate nucleus (NC), putamen, thalamus,

amygdala, and inferior temporal cortex (GTi). The BP_{ND} of these VOIs were calculated using the Lammertsma Simplified Reference Tissue Model, with the cerebellum as reference region.[29] The individual subject's receptor occupancy was defined as the percentage reduction of BP_{ND} relative to the corresponding averaged BP of the unmedicated control group according to the following equation:

$$Occupancy\ [\%] = \left(1 - \frac{BP_{Drug}}{BP_{Control}}\right) \times 100$$

Ziprasidone Occupancy After Single-Dose Treatment

The study was approved by the Joint Committee on Clinical Investigation and the Radiation Safety Committee. All participants gave written informed consent to be included. Administration of [^{11}C]RAC to the subjects was approved by the Food and Drug Administration.

Subjects

Four male healthy subjects (23–40 years; mean, 28 years) were included. All subjects underwent physical and mental-state examinations. No subjects with any mental and neurological disorder or relevant somatic diseases were included for participation in the single-dose study. Subjects had not recently taken medication with actions on the central nervous system. Each subject underwent 2 pairs of [^{11}C]RAC scans. A first baseline [^{11}C]RAC scan was followed by a second [^{11}C]RAC scan initiated 3 hours after administration of ziprasidone (40 mg, orally). At least 2 weeks later, the subjects underwent a second baseline scan followed by another [^{11}C]RAC scan 3 hours after haloperidol ingestion (7.5 mg, orally). The 3-hour interval was chosen because this duration approximates the t_{max} values of ziprasidone and haloperidol in young male subjects.[30,31]

Radiochemistry and Data Acquisition

The [^{11}C]RAC radiochemical synthesis was performed as previously described.[32,33] Images were acquired on a GE 4096+ PET system (General Electrics Medical System, Milwaukee, Wis) in 2D mode. Data acquisition consisted of a series of 58 time frames (8 × 15 and 16 × 30 seconds and 6 × 1, 6 × 2, 11 × 4, and 11 × 6 minutes), resulting in a total scanning time of 90 minutes. Attenuation correction of emission data was carried out using a transmission scan obtained in 2D mode. [^{11}C]raclopride was injected intravenously as a bolus into a cubital vein over approximately 30 seconds. Venous blood samples were taken at intervals up to 8 hours after drug treatment for assays of serum drug and prolactin concentrations.

Image and Data Analysis

During [^{11}C]RAC recordings, head position was held in place using a thermoplastic mask with laser alignment. Slight movements between baseline and blocked [^{11}C]RAC scans were corrected by coregistration of the summed images. Data analysis and receptor occupancy calculations were performed as described previously by Yokoi et al.[33] For this

TABLE 1. Individual Data of the Patients Receiving Stable Doses of Ziprasidone

				Interval: Last Dose–PET		C_{zip}, ng/mL		D_2/D_3-Receptor Occupancy, %			
No.	Sex	Age, yrs	Ziprasidone Daily Dose, mg	Long, h	Short, h	Morning*	PET Scan (Average)	NC	Putamen	Thalamus	GTi
1	m	40	200	33.50	—	—	69	50	52	60	66
2	m	34	200	18.25	—	219	205	84	82	82	77
3	f	44	160	24.00	—	60	54	22	17	21	42
4	m	37	140	22.75	—	122	84	64	57	74	75
5	m	21	160	18.00	—	98	101	57	52	52	62
6	m	20	120	21.75	—	162	133	78	75	79	70
7	f	22	120	18.75	—	—	106	73	72	74	73
8	f	42	120	14.25	—	29	35	33	29	48	53
9	m	32	160	—	5.25	119	288	84	84	90	85
10	m	25	160	—	8.75	49	60	52	49	71	71
11	m	25	160	—	9.75	48	45	43	43	49	60
12	m	24	160	—	4.75	47	216	84	80	78	80
13	f	30	100	—	6.50	—	83	58	57	60	61
14	m	30	100	—	6.75	18	70	61	58	70	64
15	m	32	80	—	10.00	75	27	43	42	51	57
Average	—	30.5	143	21.4	7.4	87.2	105	59	57	64	66
SD	—	7.74	35.3	5.79	2.12	59.53	75.3	19.2	19.6	17.7	11.2

The long- and short-interval groups were defined by an interval between last ingestion of ziprasidone and the PET scan of more than 10 hours and less than or equal to 10 hours, respectively.

*Morning plasma levels were not generally collected at the days of PET scans.

study, receptor occupancy after haloperidol and ziprasidone treatment was defined as percentage reduction of BP_{ND} relative to the individual baseline BP estimates.

Statistical Analyses

Statistical analyses were carried out by using the commercial SPSS software (v14.0; SPSS Inc, Chicago, Ill). Unpaired *t*-tests were used to compare binding values between the treatment groups. Plasma concentrations and D_2/D_3-receptor occupancy values were analyzed by Spearman rank-order correlations and additionally fitted by nonlinear regression analysis using SigmaPlot (v9.0; Systat, San Jose, Calif) to the following equation:

$$Occupancy\ [\%] = \frac{E_{max} \times [C_{Zip}]}{EC_{50} + [C_{Zip}]}$$

where E_{max} (maximum attainable receptor occupancy) was constrained to 100%, and where C_{zip} is the mean plasma concentration of ziprasidone during the PET scan. The 95% confidence interval for the regression was calculated, as well as the 95% prediction interval, which predicts the likely range of values for the population from which the sample was drawn. Confidence and prediction intervals were calculated by error propagation using the last covariance matrix of the iterative process of the previously mentioned nonlinear regression as implemented in the LAB Fit Software V7.2.42.[34]

EC_{50}, EC_{60}, and EC_{80} are the plasma concentrations thus predicted to provide 50%, 60%, and 80% receptor occupancy. In all analyses, the 2-tailed level of statistical significance was set at $\alpha = 0.05$. For comparison of D_2/D_3-receptor occupancies after long and short intervals between the last dose and PET scan, the residuals between the observed and predicted values (nonlinear regression; vide infra) of FP displacement (OC_{err}) were compared between these groups by a 2-tailed unpaired *t*-test. Because a priori hypotheses were tested, there was no adjustment for multiple testing.

RESULTS

Injected activities of FP did not significantly differ between the ziprasidone and the control group (ziprasidone: mean, 217 MBq [SD, 42 MBq]; control: mean, 233 MBq [SD, 46 MBq]; $P = 0.439$; 2-tailed unpaired *t* test). The BP_{ND} values of untreated patients were 24.1 (SD, 3.6) in the putamen, 21.8 (SD, 3.2) in the NC, 2.4 (SD, 0.5) in the thalamus, and 0.9 (SD, 0.1) in the GTi. Corresponding values of the treated patients were 10.5 (SD, 4.7) in the putamen, 8.9 (SD, 4.2) in the NC, 0.9 (SD, 0.4) in the thalamus, and 0.3 (SD, 0.1) in the GTi. The individual and mean D_2/D_3-receptor occupancies by region are shown in Table 1. D_2/D_3-receptor occupancies in the striatal VOIs were significantly lower than in the GTi ($\Delta_{GTi\text{-}NC} = +7.4\%$, $P = 0.012$; $\Delta_{GTi\text{-}PUT} = +9.8\%$, $P = 0.003$; 2-tailed paired *t* test) and in the thalamus ($\Delta_{THA\text{-}NC} = +4.8\%$, $P = 0.020$; $\Delta_{THA\text{-}PUT} = +7.1\%$, $P = 0.0018$; 2-tailed

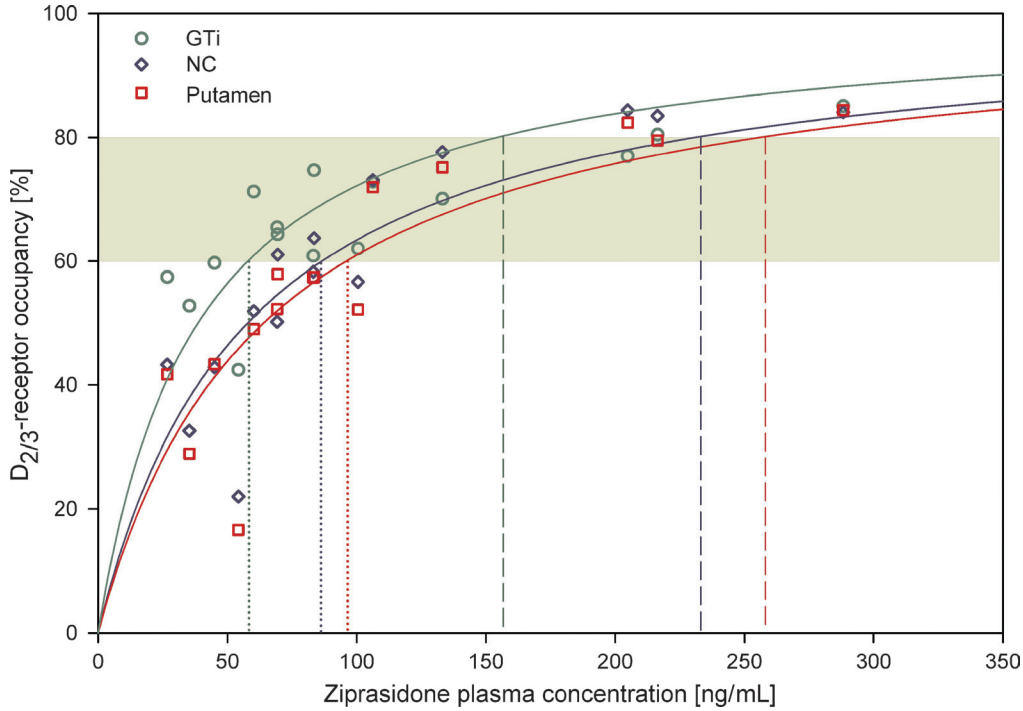

FIGURE 1. Nonlinear regression after steady-state treatment illustrating the relationship between ziprasidone plasma concentration (during the PET scan) and the D_2/D_3-receptor occupancy in the inferior temporal gyrus (green circles), the NC (blue checkers), and the putamen (red squares), according to the Michaelis-Menten equation [Occ = ($E_{max} \times C_{zip}$) / $EC_{50} + C_{zip}$); B_{max} fixed at 100%]. The yellow band depicts the clinically important area of receptor occupancies between 60% and 80%. The dotted drop lines mark the concentrations needed to cause 60% FP displacement in the corresponding central structure. The dashed drop lines mark the concentrations needed to cause 80% occupancies (data based on the nonlinear regression model).

TABLE 2. Correlation Between D_2/D_3-Receptor Occupancy and Ziprasidone Plasma Concentration

Region	D_2/D_3-Receptor Occupancy, mean (SD), %	EC_{50}, ng/mL	EC_{60}, ng/mL	EC_{80}, ng/mL	C_{zip} vs D_2/D_3-Receptor Occupancy: Nonlinear Regression, R^2_{adj}; P	C_{zip} vs D_2/D_3-Receptor Occupancy: Rank-Order Correlation, r; P
NC	59.0 (19.2)	57	87	231	0.78; <0.001	0.93; <0.001
Putamen	56.7 (19.6)	64	96	256	0.73; <0.001	0.93; <0.001
Thalamus	63.8 (17.7)	45	67	180	0.53; 0.002	0.87; <0.001
Amygdala	59.3 (22.7)	57	85	226	0.71; <0.001	0.83; <0.001
GTi	66.4 (11.2)	39	58	154	0.61; <0.001	0.85; <0.001

Depicted are the rank-order correlations between the ziprasidone plasma concentration during the PET scan (C_{zip}) and the calculated FP displacement (2-tailed Spearman correlation) and the results of a nonlinear regression using the Michaelis-Menten equation with E_{max} fixed to 100%. Concentrations causing 50% (EC_{50}), 60% (EC_{60}), and 80% (EC_{80}) D_2/D_3-receptor occupancy were calculated based on the nonlinear regression model.

paired t-test). There were no significant differences between the occupancies in the GTi and the thalamus.

By design, patients in the long-interval (mean, 21.4 hours [SD, 5.8 hours]) and the short-interval (mean, 7.4 [SD, 2.1 hours]) groups differed significantly in the time delay between the ziprasidone intake and initiation of the PET scan (Δ_t: +14.0 hours, $P < 0.001$; 2-tailed unpaired t-test). Due to the intentional higher daily dosing in the patients of the long-interval group, there were no significant differences of the ziprasidone intrascan plasma concentration between the groups (long: 112.7 ng/mL [SD, 99.0 ng/mL]; short: 98.4 ng/mL [SD, 53.0 ng/mL]; $P = 0.73$; 2-tailed unpaired t-test). Neither the trough ziprasidone concentration nor the intrascan concentration correlated significantly with daily dose (C_{mor}: $r = 0.42$, $P = 0.175$; Pearson correlation). The intrascan ziprasidone concentrations correlated significantly with the D_2/D_3-receptor occupancies in all VOIs (Fig. 1). A significant correlation was also present according to the nonlinear Spearman rank-order test (Table 2).

The time interval between the time point of the last ziprasidone ingestion and the initiation of the PET scan had no effect on the relationship between D_2/D_3-receptor

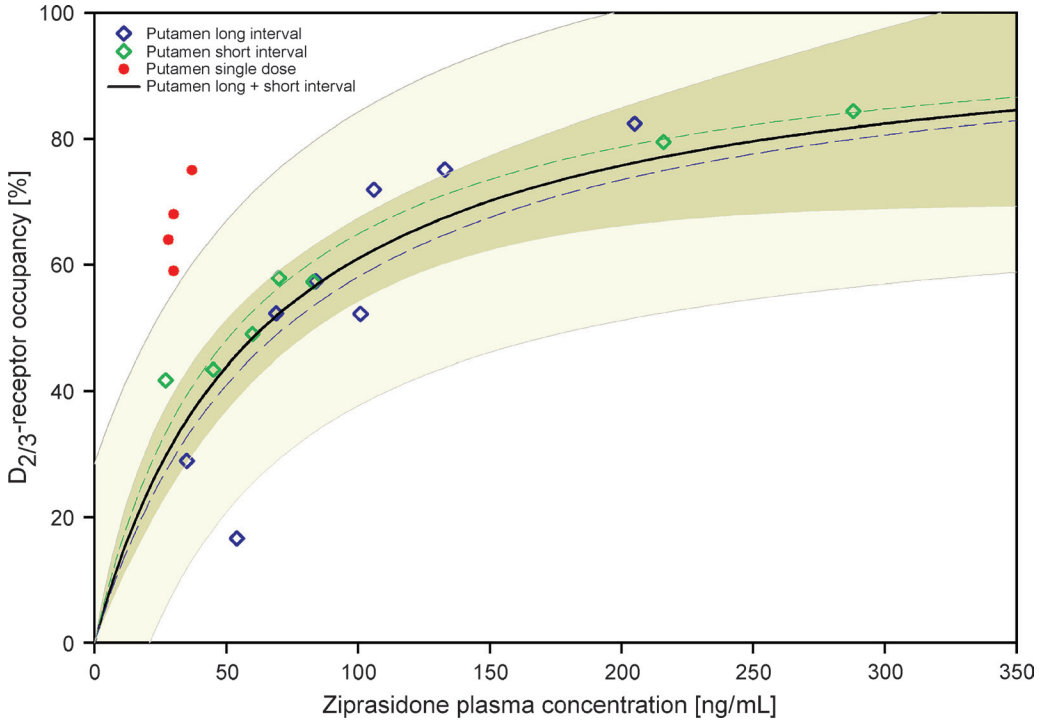

FIGURE 2. Nonlinear regression curves (exemplified for putamen) of the short-interval group (green dashed line and checkers) and the long-interval group (blue dashed line and checkers) of patients under stable ziprasidone dose do not exceed the area of the 95% confidence intervals based on the regression model (yellow area, black line: regression for the whole sample). [^{11}C]raclopride displacement of 4 healthy subjects receiving a single dose of 40 mg (red dots) is entirely outside the range of the 95% confidence intervals and also outside the 95% prediction intervals (pale yellow area).

occupancy and the corresponding ziprasidone plasma level in striatum or any other brain region (Fig. 2). There were no significant differences between measured and expected occupancy levels (OC_{err}) in any of the VOIs for the short- and the long-interval group. Consequently, there was no correlation between the time interval and OC_{err}.

Binding potentials for [^{11}C]RAC in the striatum of healthy volunteers did not significantly differ between the 2 baseline conditions (ziprasidone: 2.32 [SD, 0.28]; haloperidol: 2.45 [SD, 0.25]). Ziprasidone treatment evoked 59% to 75% D_2/D_3-receptor occupancy with serum ziprasidone concentrations ranging from 28.0 to 37.0 ng/mL. Haloperidol treatment evoked 79% to 86% D_2/D_3-receptor occupancy with serum haloperidol concentrations of 1.19 to 2.90 ng/mL. Both drugs were associated with 5- to 8-fold increases in serum prolactin concentrations.

DISCUSSION

Subchronic Dosing in Patients With Schizophrenia

Although several PET- and single-photon emission computed tomography investigations have been carried out to determine the D_2/D_3-receptor–binding profile of ziprasidone,[7–9] this is the first imaging study using a high-affinity D_2/D_3-ligand (FP) to also determine D_2/D_3-receptor occupancy at extrastriatal sites. This investigation showed that ziprasidone exerts a significantly 5% to 10% higher D_2/D_3-receptor occupancy in extrastriatal than in striatal regions. Apparently, the extent of this preferential extrastriatal binding varied over the range of plasma levels. For further analysis, the regional D_2/D_3-receptor occupancy was tested by a nonlinear regression model (based on the Michaelis-Menten equation) as a function of ziprasidone plasma levels by fitting the EC_{50} values to the measured data. In all VOIs, this model led to high adjusted coefficients of determination (R^2_{adj}), indicating excellent goodness of fits. On theoretical grounds, E_{max} was constrained to a value of 100%, exactly as in the approach taken by Mamo et al[9]; visual inspection of the fittings validates the use of this E_{max} value (Figs. 1, 2). In the striatum, the relationship between plasma ziprasidone concentration and receptor occupancy was similar to that in an earlier study using [^{11}C]RAC, in which steady-state plasma ziprasidone concentrations of 60 to 160 ng/mL were associated with 60% to 70% D_2/D_3-receptor occupancy.[9] However, due to our use of higher average dosages, and the scanning of some patients at a short interval after the last drug ingestion, we were able to investigate D_2/D_3-receptor occupancy at much higher concentrations (up to 288 ng/mL). We found that plasma levels of more than 200 ng/mL are associated with striatal receptor occupancy levels of more than 80%, a threshold that is generally associated with notable extrapyramidal side effects (EPSs).[15] In this range of plasma concentrations, a difference between striatal and extrastriatal D_2/D_3-receptor occupancy is not detectable with FP-PET. Accordingly, the most relevant difference between striatal and extrastriatal occupancy was detected at lower plasma drug concentrations (30–100 ng/mL).

As noted above, striatal D_2/D_3-receptor occupancy levels of more than 80% are considered to increase the risk of EPSs; a receptor occupancy of less than 60% bears the risk of insufficient antipsychotic efficacy.[15] Based on the regression model, we therefore calculated ziprasidone plasma levels causing 60% (EC_{60}) and 80% (EC_{80}) D_2/D_3-receptor occupancy. As a consequence of the abovementioned higher D_2/D_3-receptor binding in extrastriatal regions, the range of plasma concentrations associated with 60% to 80% receptor binding varies between the VOIs (putamen: EC_{60} = 96 ng/mL, EC_{80} = 256 ng/mL; GTi: EC_{60} = 56 ng/mL, EC_{80} = 154 ng/mL). Similar observations have been reported previously for several SGAs with moderate D_2-receptor affinity (eg, amisulpride or risperidone),[20,23,34] whereas the effect is much more pronounced for antipsychotics with very low affinities such as clozapine and quetiapine.[17,22] In contrast, it seems to be clinically meaningless or absent in examinations of antipsychotics with high affinity such as FGAs and the partial agonist aripiprazole (at least at daily doses >10 mg/d).[35–37]

However, it has to be mentioned that the observation of a preferential extrastriatal binding of SGAs with low or moderate affinity at D_2/D_3-receptors was not unequivocally reported by all authors who were involved in this topic. Frankle[38] reviewed a large number of occupancy studies in which high-affinity ligands were applied. He reported 13 studies that investigated striatal versus extrastriatal binding of SGAs. Four groups failed to find a significant elevation of extrastriatal drug binding. It is noteworthy that 3 of these 4 studies were performed by using a dual-tracer approach ([^{11}C]RAC vs [^{11}C]FLB457).[21,39,40] This may cause a bias in the results. Kessler at al,[41] using a single-tracer approach ([^{18}F]FP), found no preferential extrastriatal binding for olanzapine,[41] but for quetiapine and clozapine.[17] All mentioned negative reports suffered from low group sizes ranging from n = 1 to n = 7 per substance. Furthermore, some authors questioned the validity of the preferential extrastriatal-binding finding itself. Based on simulations for [^{11}C]-labeled ligands, these authors claimed that the methods used for BP_{ND} calculation may overestimate the BP_{ND} values in receptor-rich regions.[42] However, corresponding simulations were not performed with [^{18}F]-labeled high-affinity ligands, which permit prolonged scan duration. Another reason for a possible incongruity between striatal and extrastriatal results might be the influence of the very low concentrations of cerebellar D_2 receptors.[43] However, Kessler et al[41] compared regional BP_{ND} obtained with Logan plots using a metabolite-corrected plasma input function with those obtained with the reference region method and found a correlation coefficient greater than 0.99 with a slope of 1.0. These authors did not detect any differences between cerebellar and white matter binding in the ratio of unblocked versus blocked states, indicating a lack of discernible binding in the cerebellum. The abovementioned arguments cannot sufficiently explain why the extent of preferential extrastriatal binding is more pronounced in antipsychotics with very low D_2/D_3-receptor affinity (ie, clozapine) and is absent or clinically meaningless in substances with very high affinity (eg, aripiprazole) when using the same methodological approach and looking at suitable antipsychotic daily doses.

Taken together, preferential extrastriatal binding of ziprasidone does not seem to be solely based on methodological artifacts. Nevertheless, the impact of this difference on the "atypical" clinical properties of an antipsychotic is a matter of debate. Agid et al[40] found striatal D_2/D_3-receptor occupancy to be correlated with symptom reduction in patients treated with risperidone or olanzapine; the occupancy in extrastriatal regions failed to show such a correlation. However, we have earlier demonstrated that effective clozapine treatment resulted in less than 40% D_2/D_3-receptor occupancy in the striatum, but considerably higher levels in extrastriatal regions.[22] The finding by Agid et al[40] might have been driven by the relatively high occupancy of extrastriatal D_2/D_3 receptors by olanzapine and risperidone, even at low dose. Whereas it is impossible to detect a correlation with a parameter with low statistical variability, it is more likely to find an association of antipsychotic efficacy with a parameter with higher variability such as striatal binding. Although receptor occupancy studies have not yet conclusively clarified whether antipsychotic effects are mediated in striatal or extrastriatal (or probably both) brain regions, the extent of preferential extrastriatal D_2/D_3-receptor binding seems to be closely associated with the clinically observed extent of "atypicality."

Given that a striatal D_2/D_3-receptor occupancy of more than 80% increases the risk of EPSs and an occupancy level of more than 60% (at least in extrastriatal regions) is necessary to provide antipsychotic efficacy, present steady-state results suggest that a theoretical plasma range of 56 to 231 ng/mL of ziprasidone might be suitable to provide an optimal efficacy–side effect relationship. The lower threshold is in good agreement with the dosing guidelines based on therapeutic drug monitoring and clinical observations[44] recommending ziprasidone plasma concentrations between 50 and 120 ng/mL (trough plasma concentrations at steady state). It is notable that several subjects, although being treated with recommended daily doses of ziprasidone, had trough plasma concentrations of less than 50 ng/mL and consequently failed to exceed that 60% D_2/D_3-receptor occupancy. On the other hand, trough plasma concentrations of about 120 ng/mL may be associated with peak levels of ziprasidone of more than 230 ng/mL, which are associated with greater than 80% receptor D_2/D_3-receptor occupancy in striatal structures (eg, subject 5).

The relationship between plasma concentration and regional D_2/D_3-receptor occupancy was independent from the time interval between the last dose of ziprasidone and the PET scan. Thus, concentration/occupancy curves calculated for the subgroup of patients with short time intervals were not different from those of patients with long time intervals (Fig. 2). Obviously, the D_2/D_3-receptor occupancy is rapidly synchronizing with the daily course of ziprasidone plasma levels. Tauscher et al[45] investigated the time course of olanzapine and risperidone plasma levels relative to their D_2/D_3-receptor binding using [^{11}C]RAC-PET. These authors discuss their finding of dissociation between plasma levels and receptor binding not only on the basis of the known nonlinear plasma-occupancy relationship, but also by addressing a slow dissociation of the bound drug from the receptors and consequently discrepant plasma/tissue drug concentrations. The present investigation was not designed to directly measure the time course of D_2/D_3-receptor occupancies after the last dose of ziprasidone. However, the absence of any time effect on the concentration-occupancy relationship negates the relevance of such mechanisms in case of ziprasidone.

Single Versus Multiple Dosing

It might be predicted from the present single-dose study that 40 mg ziprasidone is an ideal daily dose for the treatment of schizophrenia. This conclusion was also drawn by Bench et al[7] from their single-dose studies with ziprasidone. However, the minimum effective dose has been found to be 120 mg/d.[46] The present investigation under steady-state conditions revealed greater than 60% occupancy of striatal receptors under clinically useful ziprasidone doses (mean, 143 mg daily). In another recent study, subchronic treatment (40–160 mg/d) evoked a mean striatal D_2/D_3-receptor occupancy of 56%.[9] Thus, results of the 2 available PET studies with single doses predict a higher D_2/D_3-receptor occupancy than the 2 studies with subchronic treatment. The occupancy values determined after single doses of ziprasidone were far outside the 95% prediction (tolerance) limits of the regression line for occupancy versus plasma concentration with chronic dosing (Fig. 2).

Although one might object that our sample size may be too modest to support our conclusions, the haloperidol results argue to the contrary. We determined a mean striatal D_2/D_3-receptor occupancy of 83% at a mean haloperidol plasma concentration of 1.77 ng/mL. This is exactly the occupancy that can be predicted from the relationship between striatal D_2/D_3-receptor occupancy and haloperidol plasma concentration determined after subchronic treatment. Fitzgerald et al[47] calculated a median effective dose (ED_{50}) of 0.35 ng/mL for haloperidol in a sample of 32 patients treated subchronically with haloperidol. Striatal D_2/D_3-receptor occupancy can be calculated from plasma levels and the ED_{50} value. Using an ED_{50} of 0.35 ng/mL, a haloperidol plasma concentration of 1.77 ng/mL (mean plasma concentration in our study) leads to a striatal D_2/D_3-receptor occupancy of exactly 83% (mean D_2/D_3-receptor occupancy in our study). Thus, for haloperidol there is a remarkable agreement between single- and multiple-dose studies with regard to the relationship between plasma concentration and striatal D_2/D_3-receptor occupancy even in a small sample. Furthermore, it has been shown both for olanzapine[48,49] and for risperidone[49,50] that receptor occupancy values assessed under subchronic treatment can be reliably predicted from single-dose studies in normal volunteers, even in samples with a size of just 3 subjects.[51]

Due to the different radioligand availabilities at the 2 study centers, the single-dose and the steady-state investigations were performed by the use of 2 different ligands (ie, [^{11}C]RAC and [^{18}F]FP). We are aware of the fact that differences in ligand affinity for D_2/D_3 receptors may cause discrepancies in observed occupancy. However, the discrepancy between the steady-state and the single-dose regimen remains present when the results of our single-dose study are compared with data obtained with the same radiotracer in patients treated subchronically with ziprasidone.[9] Although the ED_{50} value determined by these authors is somewhat

lower (33 ng/mL), the D_2/D_3-receptor occupancy found under single-dose conditions in our study is on average 17% higher than predicted by the regression calculated by Mamo et al.[9] Taken together, the differences between single-dose and steady-state results do not seem to be solely caused by the selection of radioligands.

We found similar elevations in prolactin secretion after haloperidol and ziprasidone ingestion. In contrast, Mamo et al[9] found no change in prolactin concentrations in most patients treated subchronically with ziprasidone, although plasma drug levels in their study were significantly higher than in our 4 volunteers after single-dose treatment. Thus, prolactin secretion seems to be another biomarker that is differently regulated with single and multiple ziprasidone dosing.

Bench et al[52] discussed changes in dopamine-receptor abundance and availability as confounds in the interpretation of some occupancy studies. Acute treatment with haloperidol stimulates dopamine transmission in striatum of healthy humans,[53] which might result in elevated competition from endogenous dopamine and consequent overestimation of receptor occupancy. On the other hand, ongoing antipsychotic treatment evokes D_2-receptor up-regulation,[54] which might lead to an underestimation of D_2/D_3-receptor occupation. However, these mechanisms would be of greater relevance with FGAs than with SGAs[55] and thus are unlikely to explain the present discrepancies. Apparently, incongruent occupancy results seem peculiar to a subgroup of antipsychotics, but the determining factors for this phenomenon are yet to be established. Taken together, the present incongruity of occupancy results and the abovementioned absence of any time-interval effects between last dosing and PET scan are unexpected results. It may be noteworthy that additional irregular pharmacological properties have been reported for ziprasidone.[56] Specifically, these authors found ziprasidone to have a low blood-brain permeability compared with a relatively high lipophilicity and suggested the occurrence of active transport mechanisms distinct from P450 glycoprotein.

The present results do not merely uncover some irregula pharmacological properties of ziprasidone, but have major implications for the clinical interpretation of occupancy studies. In the case of ziprasidone, single-dose PET experiments seem insufficient to predict daily doses or plasma concentrations associated with sufficient receptor occupancy for antipsychotic efficacy. Our results suggest that single-dose studies, although still very important in phase 1 drug development, may not be totally relied on for final dose selection. A combination of single-dose studies in phase 1 and multiple-dose studies in phase 2 may be more appropriate. Furthermore, the particular pharmacological profile of the investigational drug has to be taken into consideration when drawing substantial and potentially expensive conclusions for phase 3 trials.[27]

In conclusion, we demonstrate in a series of PET studies that ziprasidone is characterized by "preferential" extrastriatal D_2/D_3-receptor binding over a wide range of plasma concentrations. This feature might be partly the basis for ziprasidone's "atypical" clinical properties. Single-dose studies do not necessarily predict the doses needed for antipsychotic efficacy, which underlines the need for single- and multiple-dosing studies in drug development.

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AUTHOR DISCLOSURE INFORMATION

Dr Vernaleken has served as a consultant for Bristol-Myers Squibb. Dr Bartenstein has served as a consultant for Siemens Medical Solutions (Erlangen), Philips Electronics (Eindhoven), Novartis Pharma AG (Basel), and Schering AG (Berlin). He has received grant support from Schering AG and Novartis Pharma AG. Dr Hiemke has served as a consultant for Eli Lilly and Servier (Paris, France). He has received grant support from Pfizer, Sanofi-Aventis (Paris, France), Roche (Basel, Switzerland), and Bio-Rad Laboratories (Munich, Germany). Dr Wong has received support from the National Institutes of Health, Acadia, Avid, Dainippon Sumitomo Pharma America, Johnson & Johnson, Otsuka, Lilly, Merck, Orexigen, Philip Morris, Roche, Sanofi-Aventis, DANA Foundation, and NARSAD. Dr Gründer has served as a consultant for AstraZeneca (London, UK), Bristol-Myers Squibb (New York, NY), Johnson & Johnson (Beerse, Belgium), Otsuka (Rockville, MD), and Pfizer (New York, NY). He has served on the speakers' bureau of AstraZeneca, Bristol-Myers Squibb, Eli Lilly (Indianapolis, IN), Janssen Cilag, Otsuka, Pfizer, and Wyeth-Ayerst (Collegeville, PA). He has received grant support from Bristol-Myers Squibb, Johnson & Johnson, and Pfizer. Dr Schäfer has served as a consultant for GE Healthcare Buchler (München, Germany), Siemens Medical Solutions (Erlangen, Germany), Philips (Aachen, Germany), and Bristol-Myers Squibb (München, Germany). He has received grant support from Mediso Medical Imaging Systems (Budapest, Hungary). Mr Buchholz and Drs Janouschek, Bröcheler, Veselinovic, Landvogt, Cumming, Boy, Dr Fellows, and Dr Spreckelmeyer report no competing interests.

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