Modeling of Brain D_2 Receptor Occupancy-Plasma Concentration Relationships with a Novel Antipsychotic, YKP1358, Using Serial PET Scans in Healthy Volunteers

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YKP1358 is a novel serotonin (5-HT_{2A}) and dopamine (D_2) antagonist that, in preclinical studies, fits the general profile of an atypical antipsychotic. We conducted a D_2 receptor occupancy study with YKP1358 in healthy volunteers using positron emission tomography (PET) to measure the D_2 receptor occupancy of YKP1358 and to characterize its relationship to plasma drug concentrations. A single oral dose, parallel group, dose-escalation (100, 200, and 250 mg) study was performed in 10 healthy male volunteers with the PET radiotracer [^{11}C]raclopride. The D_2 receptor occupancy of striatum was measured pre-dose, and at 2, 5, and 10 h after YKP1358 administration. Serial blood samples were taken for measurement of plasma YKP1358 concentrations. D_2 receptor occupancy by YKP1358 increased to 53–83% at 2 h, and then decreased afterwards, ranging from 40–64% at 5 h to 20–51% at 10 h. The YKP1358 dose–plasma concentration relationship exhibited extensive variability, but there was a good relationship between plasma concentrations and D_2 receptor occupancy that was well predicted by a sigmoid E_{max} model using nonlinear mixed effects modeling. To our knowledge, this is the first study in which the relationship between plasma concentration and the biomarker of D_2 receptor occupancy was modeled using nonlinear mixed effects modeling. It is anticipated that these results will be useful in estimating for subsequent studies the initial doses of YKP1358 required to achieve a therapeutically effective range of D_2 receptor occupancy.

During clinical development of an antipsychotic drug, it is difficult to define a clinically relevant dosage range from early studies involving healthy volunteers, because most healthy volunteers do not tolerate doses that are effective for schizophrenic patients. There are also few adequate pharmacodynamic biomarkers for antipsychotics. Therefore, pharmacokinetic–pharmacodynamic (PK–PD) modeling, using functional imaging methodology such as positron emission tomography (PET), may be a very useful tool for early antipsychotic development, especially in suggesting initial doses for further clinical studies.

All antipsychotic drugs currently used in schizophrenia treatment act to some extent by blocking dopamine neurotransmission. Compared with typical antipsychotic drugs, atypical antipsychotic drugs have relatively lower affinity for the dopamine receptor and a higher affinity for the serotonin receptor. However, dopamine receptor occupancy still plays a vital role in their antipsychotic effects.[1]

YKP1358 (C21H24CIFN2O3) is a novel antipsychotic currently in clinical development. YKP1358 fits the general profile of an atypical neuroleptic in preclinical studies because it is more potent as a 5-HT_{2A} antagonist than a D_2

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antagonist (K_i values: 85, 91, and 0.83 nM for D_{2L}, D_{2S}, and 5-HT_{2A}, respectively; unpublished data, SK Corporation, Seoul, Korea). YKP1358 also is similar to risperidone in that it increases dopamine and acetylcholine release in the medial prefrontal cortex and dopamine in the nucleus accumbens, which may enable the compound to improve negative symptoms and cognition in schizophrenia.[2]

PET studies have shown a relationship between striatal D_2 receptor occupancy and clinical effect for most typical antipsychotic medications, with clinical efficacy occurring when at least 60% of striatal D_2 receptors are occupied, whereas extrapyramidal side effects occur at D_2 receptor occupancy above 80%.[3] For most atypical antipsychotics such as risperidone and olanzapine, the relationship between dopamine D_2 receptor occupancy studied in human subjects with PET and clinical effects, including both therapeutic response and extrapyramidal side effects, is similar to that found with typical antipsychotic drugs.[4,5] However, clozapine and quetiapine show relatively low dopamine D_2 receptor occupancy (30–60%), even at the higher end of their therapeutic dosing range.[5,6]

The study described in this report was conducted in healthy male volunteers using serial PET scanning to evaluate the time course of D_2 receptor occupancy after YKP1358 single-dose administration and to characterize the relationship between D_2 receptor occupancy and plasma drug concentrations.

RESULTS

PK profiles

Individual plasma concentration–time profiles generally fit a three compartment model with first-order absorption (**Figure 1**). The mean terminal elimination half-life ($t_{1/2}$) at the 100, 200, and 250 mg dose levels was 5.58, 5.41, and 6.55 h, respectively. The coefficient of variation (CV) of the elimination half-life ranged 25–44%, whereas C_{max} (CV% range 37–120%) and $AUC_{0-\infty}$ (CV% range 29–52%) showed relatively large inter-subject variability (**Table 1**). In addition, mean C_{max} and $AUC_{0-\infty}$ at the 200 mg dose level were higher than those at the 250 mg dose level. Therefore, statistically significant linearity was not shown for C_{max}, and only borderline significance was shown for $AUC_{0-\infty}$ ($r^2 = 0.34$, $P = 0.076$, **Table 1**), although statistical significant difference was not shown in dose-normalized PK parameters with Kruskal–Wallis test (data not shown).

D_2 receptor occupancy

When D_2 receptor occupancies were examined with PET scan at 2, 5, and 10 h after single oral doses (**Figure 2**), they ranged 53–83% at 2 h among all the dose groups, and decreased to 40–64% at 5 h and 20–51% at 10 h (**Figure 3a** and **b**). D_2 receptor occupancies exhibited similar values in the putamen and caudate nucleus ($P = 0.091$, two-sided paired t-test). Mean D_2 receptor occupancy was higher in the 200 mg than in the 100 mg group, and was also higher in the 200 mg than in the 250 mg group at all three time points, which can be explained by the higher plasma concentrations shown in the 200 mg group.

D_2 receptor occupancy was well explained by plasma concentrations (**Figure 4a** and **b**), but correlation coefficients could not be calculated because this was a nonlinear regression. The CV of D_2 receptor occupancy ranged 4–12% at 2 h, although displaying larger CV values at later time

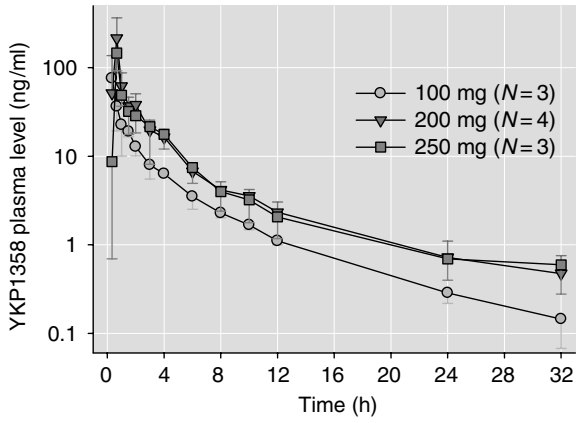

Figure 1 Plasma concentration–time profiles of YKP1358 after single oral dose administration (arithmetic mean ± SD, semilog scale).

Table 1 Pharmacokinetic parameters of YKP1358 calculated by non-compartment analysis

Parameters		C_{max} (ng/ml)			$AUC_{0-\infty}$ (ng × h/ml)		
Group	N	Arithmetic mean	SD	CV (%)	Arithmetic mean	SD	CV (%)
100 mg	3	94.0	113.1	120.3	110.4	57.8	52.4
200 mg	4	236.3	132.7	56.2	266.0	77.5	29.1
250 mg	3	146.3	54.0	36.9	217.2	65.6	30.2
r^2[a]			0.08			0.34	
P-value[a]			0.421			0.076	

C_{max}, maximum plasma concentration; $AUC_{0-\infty}$, area under plasma concentration–time curve from time 0 to infinity; CV, coefficient of variation; r^2, coefficient of determination. [a]Linear regression applied to dose and pharmacokinetic parameters. C_{max}: F=0.72, df=9; and $AUC_{0-\infty}$: F=4.16, df=9.

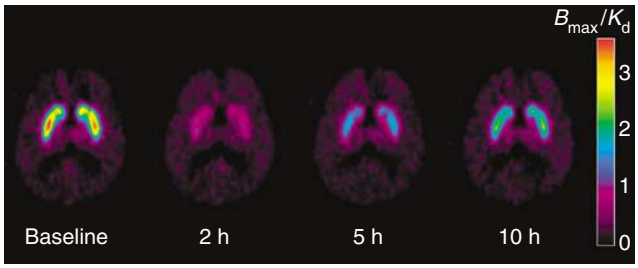

Figure 2 Serial brain PET images showing striatal dopamine D_2 receptor occupancy (parametric images of binding potential generated using Logan graphical analysis) after YKP1358 single-dose administration in a typical subject. B_{max}: density of receptor; K_d: dissociation constant; B_{max}/K_d: binding potential.

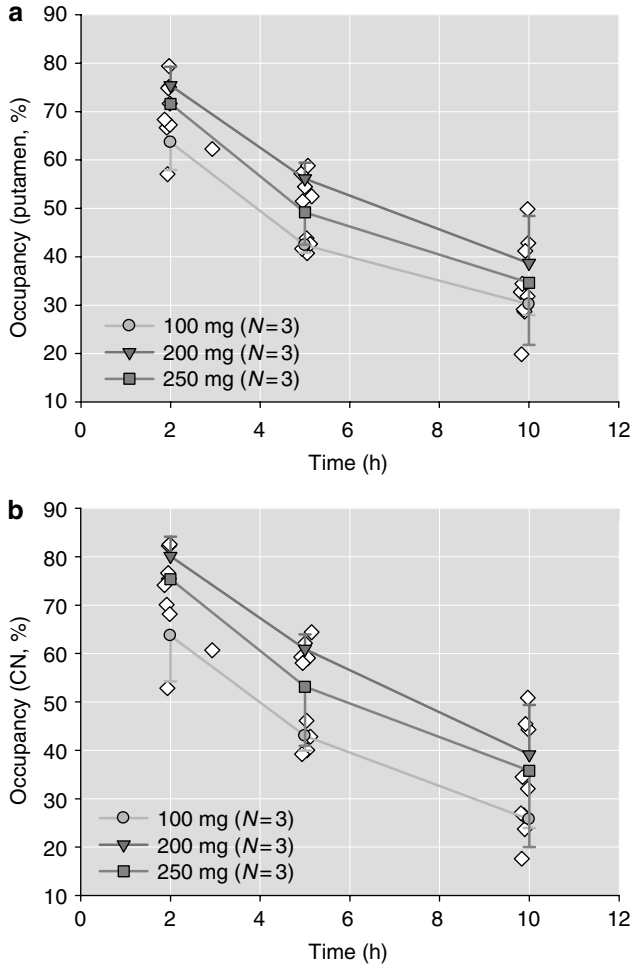

Figure 3 D_2 receptor occupancy–time profiles of YKP1358 after single oral dose administration (arithmetic mean ± SD). (**a**) Putamen and (**b**) caudate nucleus. Open diamonds (◇) represent individual subjects. One subject in the 250 mg group is not included for calculating the mean value at 2 h post-dose ($N = 2$) because the PET scan was performed 1 h later than scheduled. CN = caudate nucleus.

points, which were 3–19% at 5 h and 5–36% at 10 h after dosing.

Plasma concentration and D_2 receptor occupancy modeling

The relationship between plasma concentration and D_2 receptor occupancy was analyzed with a sigmoid E_{max} model (**Figure 4a** and **b**, and **Table 2**). E_{max} values approached 100% occupancy in both the putamen and caudate nucleus, and EC_{50} values were 10.1 and 9.4 ng/ml, respectively. As the respective Hill coefficients were 0.59 and 0.69, the graphs showed relatively flat PD features. The predicted effect site concentrations and D_2 receptor occupancy are displayed in **Figure 4c** and **d**. Mean equilibrium rate constant (k_{e0}) and equilibrium half-life were 1.92–2.02 h^{-1} and 20.6–21.7 min, respectively (**Table 2**).

DISCUSSION

In this study, we assessed the D_2 receptor occupancy of YKP1358 and its relationship with plasma concentrations in healthy volunteers. When D_2 receptor occupancies were examined with serial PET scanning at 2, 5, and 10 h after single oral administration, they ranged from 53% to 83% at 2 h in all three dose groups, and then declined over time. Because mean D_2 receptor occupancies at 10 h post-dosing were below 40% in both the 200 and 250 mg groups, the effective doses in patients are predicted to be greater than 250 mg twice a day.

Mean C_{max} and $AUC_{0-\infty}$ at the 200 mg dose level were higher than those at the 250 mg dose level, which was thought to reflect large intersubject PK variability and the fact that only small numbers of subjects were studied. Although D_2 receptor occupancies at the 200 mg dose were higher than those at the 250 mg dose, this was also the case for the plasma concentrations, which reflected a closer and more direct relationship between the D_2 receptor occupancies and the plasma concentrations rather than the nominal dose. Therefore, the plasma concentrations of YKP1358 are a more predictable marker of D_2 receptor occupancy than the dose *per se*. This is consistent with the recent study with the atypical antipsychotic ziprasidone by Mamo *et al.*[7]

We also modeled the relationship between plasma concentrations and D_2 receptor occupancy using a population PK–PD approach with NONMEM. To our knowledge, this is the first study in which the relationship between plasma concentration and D_2 occupancy was analyzed using nonlinear mixed effects modeling. Nonlinear mixed effects modeling is considered the "gold standard" in population PK–PD modeling utilizing sparse samples.[8,9] Despite a small sample size and only three points of D_2 receptor occupancy per subject, it was possible to obtain typical PK and PD parameters as well as individual values, and the D_2 receptor occupancy of YKP1358 was well predicted by a sigmoid E_{max} model. In this model EC_{50} was about 10 ng/ml, so it was predicted that if plasma concentrations exceeded 10 ng/ml, receptor occupancy would be greater than 50%. As most D_2 antagonists exhibit therapeutic effects at 60–80% D_2 occupancy,[3] the expectation is that doses of YKP1358 will need to maintain plasma concentrations greater than 10–15 ng/ml during most of the dosing interval in order to achieve therapeutic efficacy (**Figure 4a–d**).

In a recent study with olanzapine and haloperidol, Zipursky *et al.*[10] suggested that the atypical antipsychotic

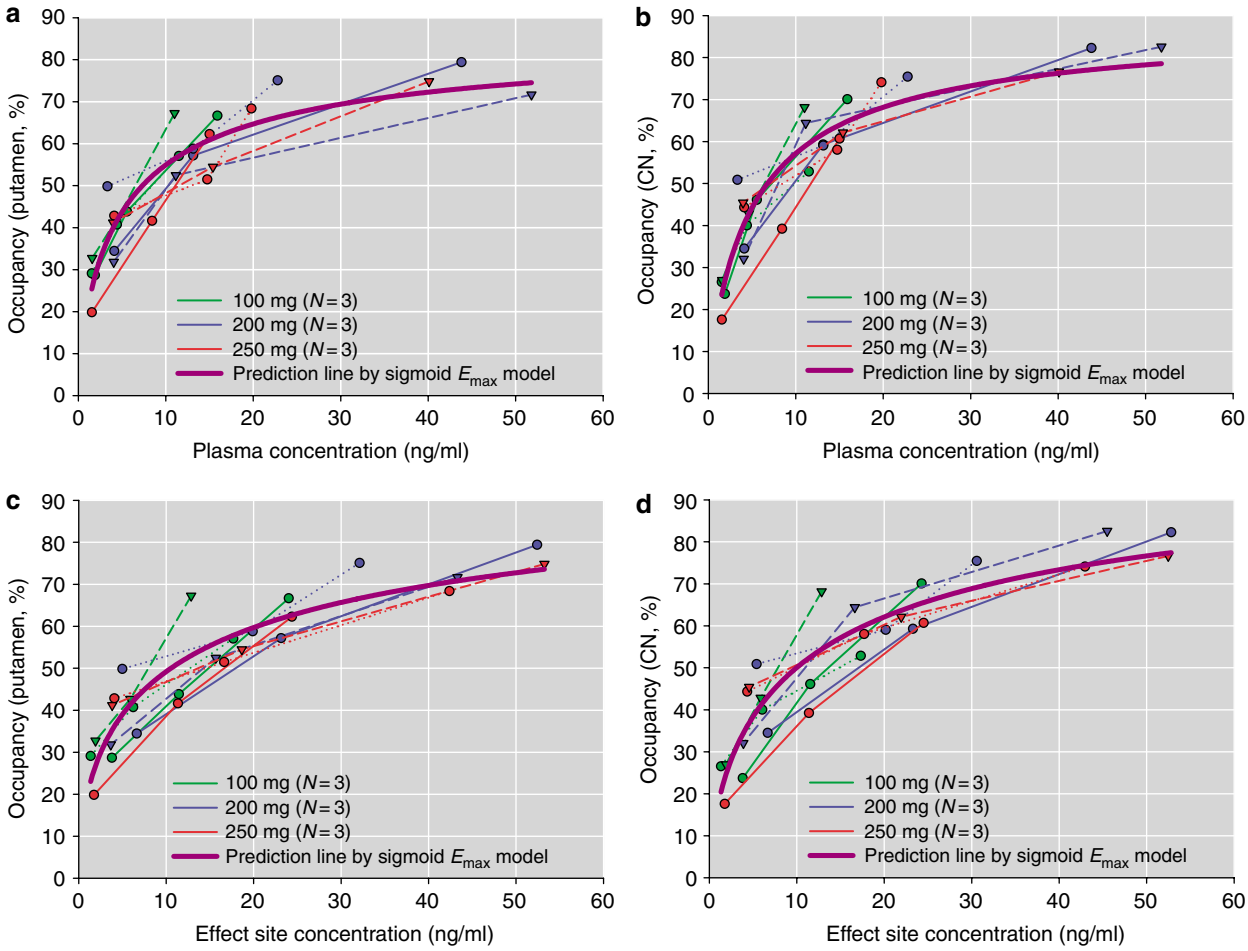

Figure 4 (**a** and **b**) D_2 receptor occupancy and plasma concentration or effect site concentration (**c** and **d**) profiles and prediction lines by sigmoid E_{max} model. Effect site concentrations were predicted by nonlinear mixed effects modeling. CN = caudate nucleus.

olanzapine exhibited therapeutic effects when D_2 receptor occupancy exceeded 70%. This is similar to the occupancies at a low dose of a typical antipsychotic, haloperidol. Nyberg *et al.*[11] also determined the minimal effective dose of risperidone in schizophrenic patients using D_2 receptor occupancy detected with PET. However, the atypical antipsychotics clozapine and quetiapine are effective with lower values of D_2 receptor occupancies, which are about 30–60%.[5,6,12] Also, the partial dopamine agonist aripiprazole has been shown to possess efficacy at more than 80–90% occupancy without significant extrapyramidal side effects.[13] At present, it is uncertain whether the binding of YKP1358 to D_2 receptors results in full antagonism or combined partial agonism. Therefore, although we can predict D_2 receptor occupancy of YKP1358 in schizophrenic patients using the plasma concentration, the therapeutic range of D_2 receptor occupancy in patients could differ from typical "atypical" antipsychotics, and further clinical evaluation is needed to confirm the PK–D_2 occupancy–therapeutic effect relationship of YKP1358 in patients.

There are several limitations of the current study that suggest caution in how these results are interpreted. Despite using mixed effects modeling, some bias could still be present because of the small number of subjects. Second, plasma kinetics and D_2 receptor occupancy profiles of repeated drug administration to schizophrenic patients might exhibit different profiles, because this is a single dose study in healthy volunteers. However, our PK results indicate that repeated dosing is not likely to result in a large accumulation of YKP1358 plasma concentrations. In addition, the effect site concentrations of YKP1358 predicted by modeling were not greatly different from the time courses of the plasma concentrations, as reflected in the short equilibrium half-life. However, the effect site concentration, *i.e.*, the concentration at the molecular target in the human brain, might differ from the plasma concentration for many antipsychotics.[14] In fact, Tauscher *et al.*[15] suggested that there are significant dissociations between brain and plasma kinetics with the atypical antipsychotics olanzapine and risperidone. Nevertheless, as they only observed that time courses of plasma concentration and D_2 receptor occupancy differ, no relationship was explicitly demonstrated. In this study, it was possible to analyze the nonlinear relationship between the plasma concentration and D_2 receptor occupancy of YKP1358 using

Table 2 Final model estimates of pharmacokinetics and pharmacodynamic parameters

Parameter	*Mean*	*SD*	*Parameter*	*Mean*	*SD*
Pharmacokinetic parameters[a]					
CL (l/h)	1040	181	V_2 (l)	2020	
V_1 (l)	665	911	Q_3 (l/h)	254	
k_a (h^{-1})	7.43		V_3 (l)	2030	629
Q_2 (l/h)	2840		Absorption lag (h)	0.380	0.169

Pharmacokinetic–pharmacodynamic parameters[b]

	Putamen		*Caudate nucleus*	
Parameter	*Typical value*	*SE*	*Typical value*	*SE*
E_{max}[c] (%)	100		100	
EC_{50} (ng/ml)	10.1	1.04	9.40	1.15
Hill coefficient	0.594	0.0522	0.685	0.0714
k_{e0}	2.12	0.392	1.92	0.657

CL, apparent clearance from central compartment; EC_{50}, plasma concentration needed to obtain half of E_{max}; E_{max}, maximum occupancy; k_a, absorption rate constant; k_{e0}, equilibrium rate constant; Q_2, intercompartmental clearance between central and rapidly equilibrating peripheral compartment; Q_3, intercompartmental clearance between central and slowly equilibrating peripheral compartment; SD, standard deviation of individual values; SE, standard error of mean for estimation; V_1, central volume of distribution; V_2, rapidly equilibrating peripheral compartment; V_3, slowly equilibrating peripheral compartment. [a]Pharmacokinetic (PK) parameters are presented as means and standard deviations of individual estimates because these fixed values were used in the PK-pharmacodynamic (PD) model. Only four PK parameters have significant inter-individual variability so SDs of these values only are presented. [b]PK–PD parameters are presented as typical values and standard errors of population estimates. [c]E_{max} value was fixed to 100% in the PK–PD model.

modeling, and D_2 receptor occupancy was found to be well predicted by plasma concentration using population PD parameters such as E_{max} and EC_{50}. Finally, the present data do not address the issue of 5-HT_2 antagonism. Although the importance of YKP1358's effect on D_2 receptors cannot be denied, 5-HT_2 antagonism also may have a critical influence on the response of atypical antipsychotics.

The purpose of this study was to obtain and utilize PK–PD information in a timely manner in order to guide decisions for further clinical study designs rather than focusing on the modeling process itself. This was based on limitations in the drug development time frame and also the small number of study subjects. This study has several implications. First, it demonstrates that YKP1358 possesses potential antipsychotic effects based on the D_2 receptor occupancy data. Second, considering the D_2 receptor occupancy data, effective doses in patients are predicted to be greater than 250 mg twice a day. In addition, compared to previous studies, this study shows that, by means of PK–PD modeling, D_2 receptor occupancy for certain drugs can be described more accurately using plasma levels than nominal doses *per se*.

METHODS

Subjects. Ten healthy non-smoking Korean male volunteers (age: 26.0 ± 4.9 years; weight: 70.2 ± 6.3 kg, mean $\pm$ SD) were enrolled in the study. None showed any abnormalities on physical examination, vital signs, laboratory tests (including hematology, blood chemistry, and urinalysis), or 12-lead electrocardiograms, and none had any relevant medical, neurological, or psychiatric disorders. All of the subjects denied any medication use within the 4 weeks before the study, and this was confirmed by urine drug screening using REMEDi HS (Bio-Rad Laboratories, Hercules, CA). Written informed consent was obtained from all subjects after the study procedures had been fully explained. The study was approved by the Institutional Review Board of Seoul National University Hospital (SNUH).

Study design. This was an open, parallel group study, with each dose group consisting of three subjects. Subjects received a single dose of YKP1358 (100, 200, or 250 mg). PET scans were not performed in one subject in the 200 mg group owing to akathisia after dosing, and only PK and safety were assessed after re-confirming the subject's consent. One subject was added to the 200 mg group in order to replace the drop-out subject and evaluate D_2 receptor occupancy. All the other subjects completed the study in accordance with the protocol and were analyzed for PK and PD.

After screening evaluation, baseline magnetic resonance (MR) imaging and PET scans were performed the day before drug administration. After overnight fasting for 12 h, YKP1358 was administered at approximately 0800 h. PET scans were performed at 2, 5, and 10 h after dosing considering previous human PK data, and blood samples for PK evaluation were collected at 0 (pre-dose), 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, and 32 h after dosing.

Assay of YKP1358 levels in plasma. Following liquid–liquid extraction using methyl-*t*-butyl ether, plasma concentrations of YKP1358 were measured by high-performance liquid chromatography (Agilent 1100 series, Agilent Technologies, Wilmington, DE) with tandem mass spectrometry (API 3000, Applied Biosystems/MDS Sciex, Toronto, Canada). Chromatographic separation was achieved under gradient conditions on a Luna C_{18} column (50×2.0 mm, 3 μM; Phenomenex, Torrance, CA) with a mobile phase consisting of 10 mM ammonium formate and acetonitrile. The mass spectrometry/mass spectrometry system was operated using an electrospray in positive ionization mode. For YKP1358 and the internal standard, YKP1683, the precursor-to-product ion reactions

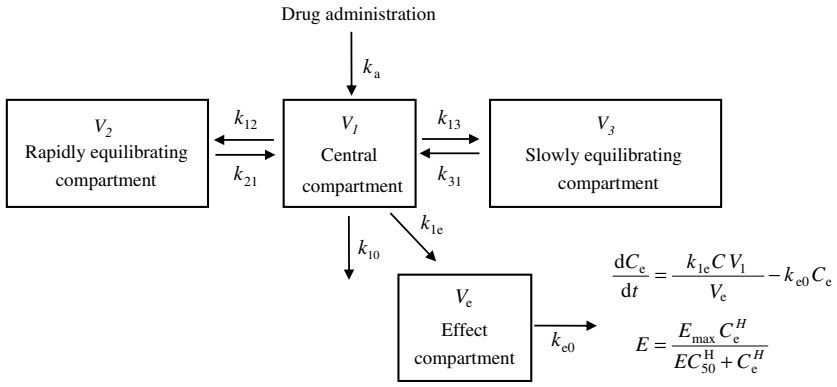

Figure 5 Pharmacokinetic-pharmacodynamic (PK–PD) model of YKP1358.

monitored were *m/z* 371.2→310.3 and 387.2→355.0, respectively. The retention times for YKP1358 and YKP1683 were 3.1 and 3.8 min, respectively, and the lower limit of quantification for YKP1358 was 0.2 ng/ml. The day-to-day CV was 6.4% at 0.6 ng/ml, 4.6% at 10 ng/ml, and 4.9% at 80 ng/ml ($N = 6$).

Synthesis of [^{11}C]raclopride. [^{11}C]raclopride was prepared in the Department of Nuclear Medicine, SNUH, according to a published method.[16] The elimination of half-life of [^{11}C]raclopride is known to be approximately 20 min.[17] [^{11}C]carbon dioxide was produced by 13 MeV proton irradiation of nitrogen gas using a TR13 cyclotron. [^{11}C]carbon dioxide was recovered from the cyclotron and trapped by 0.2 ml of 0.2 M lithium aluminum hydride solution in tetrahydrofuran. The captured ^{11}C activity was eluted with 1 ml of hydriodic acid into a preheated (110°C) glass tube. [^{11}C]methyl iodide was distilled and passed through a preheated (220°C) silver triflate column to produce [^{11}C]methyl triflate. [^{11}C]methyl triflate was passed through a high-performance liquid chromatography loop containing 1 mg of raclopride in 100 μl of cyclohexanone. [^{11}C]raclopride was purified using a preparative high-performance liquid chromatography (BioRad Rsil C18HL column, 4.6 × 150 mm, 30% ethanol in water) and collected in a 20 ml vial. The retention time of [^{11}C]raclopride was 5.2 min and the radiochemical purity was >99%. Purified [^{11}C]raclopride was analyzed using an analytical high-performance liquid chromatography (C18 column, 30% acetonitrile) equipped with an ultraviolet detector and radioisotope detector. Specific activity of [^{11}C]raclopride was calculated from the peak area of ultraviolet and injected radioactivity.

PET scan procedures. All PET scans were acquired with an ECAT EXACT 47 scanner (Siemens-CTI, Knoxville, TN), which had an intrinsic resolution of 5.2 mm full width at half maximum and images of 47 contiguous planes with thicknesses of 3.4 mm simultaneously for a longitudinal field of view of 16.2 cm. Before administration of [^{11}C]raclopride, transmission scanning was performed using three Ge-68 rod sources for attenuation correction. Dynamic 3D emission scans (15 s × 8 frames, 30 s × 16, 60 s × 10, 240 s × 10) were initiated simultaneously with a bolus injection of 370–740 MBq [^{11}C]raclopride (in less than 10 ml normal saline containing 10% ethanol) and continued for 60 min. PET was measured at 2, 5, and 10 h after dosing, but the scan of one subject in the 250 mg group scheduled at 2 h was delayed by 1 h owing to delay in raclopride synthesis. Transaxial images were reconstructed by means of a filtered back-projection algorithm employing a Shepp–Logan filter with a cutoff frequency of 0.3 cycles/pixel as 128 × 128 × 47 matrices with a size of 2.1 × 2.1 × 3.4 mm.

D_2 receptor occupancy measurement. To permit accurate delineation of the brain regions for data analysis, static PET images obtained by summing all the dynamic image frames were co-registered with the MR images from the same individual. MR imaging scans were made with a GE Signal 1.5-T scanner. The image was acquired by using a conventional T1 localizing scan and a fast spin echo sequence with a 3 mm slice thickness. The MR imaging scan of each subject was co-registered to his PET scan with SPM2. The region of interest used in the analysis of D_2 receptor occupancy was the striatum (putamen and caudate nucleus), with the cerebellum used as a reference region.[18] The regions of interest were drawn on T1 MR images by a single rater on 10 axial slices for the cerebellum and striatum. The regions of interest were then transferred to the dynamic PET images and a time activity curve was generated and used in the analysis.

A three-compartment model was employed for kinetic analysis of the binding of [^{11}C]raclopride with the dopamine D_2 receptor.[19] D_2 binding potential ($BP = B_{max}/K_d$; B_{max}: receptor density; K_d: equilibrium association constant) in the striatum was calculated using a simplified reference tissue model.[20] Receptor occupancy by YKP1358 was calculated as the percentage reduction of receptor binding potential with drug treatment compared to baseline, as shown in the following equation:[21]

$$\text{Occupancy}\,\% = \frac{BP_{baseline} - BP_{drug}}{BP_{baseline}} 100$$

PK and PD modeling. Individual PK parameters were calculated by a non-compartmental method using WinNonlin (version 5.0, Pharsight , CA). $AUC_{0-\infty}$ was calculated using the linear-up and log-down trapezoidal method in plasma concentration–time curves. Actual measured values were taken for C_{max}. For further modeling, individual and population PK parameters such as volume of distribution and clearance were obtained by non-linear mixed effects modeling using NONMEM (version V, level 1.1, GloboMax, Ellicott City, MD). A proportional error model combined with fixed additive error (lower limit of quantification/2) and the first-order method was used for the PK model, and the ADVAN12 subroutine was used because a three-compartment model was better fitted. During model building, a reduction in the minimum value of the objective function of at least 3.84 ($\alpha = 0.05$) after incorporating a single parameter was considered statistically significant.

The relationship between plasma concentration and D_2 receptor occupancy was analyzed with an indirect link model that included an effect compartment (ref. 22, **Figure 5**). In the model development step, including an equilibrium rate constant (k_{e0})

lowered the minimum value of the objective function significantly. Individual PK parameters were fixed in the PK–PD model. The ADVAN6 subroutine and an additive error model was used for the PK–PD model with the first-order conditional estimation method. The effect site concentrations and PD parameters such as E_{max} (maximum occupancy) and EC_{50} (plasma concentration needed to obtain half of E_{max}) were obtained from the following equation:

$$\text{Occupancy}\,\% = \frac{E_{max} \times Ce^{H}}{EC_{50}^{H} + Ce^{H}},$$

where Ce is the effect site concentration and H is the Hill coefficient.

Statistical analysis. Linear regression was applied to the dose and PK parameters (C_{max} and $AUC_{0-\infty}$) to evaluate whether YKP1358 demonstrates dose-independent (*i.e.*, linear) PK. Two-sided paired *t*-tests were used to compare the occupancy of the putamen with that of the caudate nucleus. Kruskal–Wallis test was used for comparison of the dose-normalized C_{max} and $AUC_{0-\infty}$. S-PLUS (version 6.0, Insightful Corp., Seattle, WA) was used for statistical analysis and the level of significance used was 0.05.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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