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ORIGINAL INVESTIGATION

# The use of healthy volunteers instead of patients to inform drug dosing studies: a [$^{11}$C]raclopride PET study

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**Abstract**
*Rationale* Receptor occupancy study has been performed to evaluate pharmacokinetic profiles in new antipsychotic drug development. While these findings highlight the value of positron emission tomography (PET) for dose-finding study, what is unclear is if it is necessary to conduct these studies in patients with schizophrenia or whether studies in healthy volunteers are adequate.
*Objectives* To determine if it is necessary to conduct dopamine receptor occupancy studies in patients with schizophrenia or whether studies in healthy volunteers are adequate for dose-finding study, we compared the concentration–occupancy relationship in terms of $EC_{50}$ between patients and healthy volunteers.
*Methods* Ten healthy volunteers and eight patients with schizophrenia participated in the study. We measured dopamine receptor occupancy using [$^{11}$C]raclopride PET and plasma concentration of YKP1358, a novel antipsychotic drug under clinical development, at a number of time points after the administration of YKP1358. Pharmacokinetic data including area under the plasma concentration versus time curve, elimination half-life, maximum observed plasma concentration, and the time to reach the maximum observed plasma concentration were obtained. We explored the relationship between plasma concentration and dopamine $D_2$ receptor occupancy using $E_{max}$ model and calculated $EC_{50}$.
*Results* The elimination half-life was longer in healthy volunteers than in patients. Other pharmacokinetic parameters were not significantly different between two groups. The $EC_{50}$ was 7.6 ng/ml (95% confidence interval (CI) 6.2–9.0) in healthy volunteers and 8.6 (95% CI 7.4–9.9) in patients.

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*Conclusions* The antipsychotic concentration–occupancy relationship in patients can be estimated from the $EC_{50}$ data of healthy volunteers.

**Keywords** Antipsychotics · Drug development · Biomarker · Positron emission tomography · Dopamine D2 receptor · Subject selection

## Introduction

The low productivity and escalating cost of new drug development have been documented extensively over the past several years. This is particularly the case for CNS disorders such as schizophrenia where the blood–brain barrier further complicates pharmacokinetic (PK) and pharmacodynamic (PD) analysis of promising new compounds (McGuire et al. 2008; Pien et al. 2005). Positron emission tomography (PET) can provide PK and PD data to inform the dose and dosing schedule of drugs and compounds in development. This approach has been used to investigate antipsychotic drug treatment of schizophrenia (Howes et al. 2009; Kegeles et al. 2008).

In particular, [$^{11}$C]raclopride PET is a useful method for measuring the dopamine $D_2$ receptor occupancy induced by antipsychotic drugs. Dopamine $D_2$ receptor occupancy is a meaningful biomarker in that it reflects the antipsychotic action at the target site in the brain and predicts both the clinical response to antipsychotic drugs and the emergence of drug side effects (Kapur et al. 2000). The importance of measuring the end organ action of a novel drug is further emphasized by studies showing wide discrepancy between the time courses of drug concentration in plasma and drug dopamine receptor occupancy in the brain (Catafau et al. 2008; Mamo et al. 2008; Tauscher et al. 2002).

PET has been used to evaluate a number of antipsychotic drugs in development to determine the $EC_{50}$ (the plasma drug concentration associated with 50% occupancy of dopamine receptors in the $E_{max}$ model; receptor occupancy=100%×drug concentration/($EC_{50}$+drug concentration)) and aid the determination of dose and dosing interval (Arakawa et al. 2008; Bench et al. 1993; Mamo et al. 2007; Vernaleken et al. 2008). While these findings highlight the value of PET to aid the evaluation of novel antipsychotics, what is unclear is if it is necessary to conduct these studies in patients with schizophrenia or whether studies in healthy volunteers are adequate. There are obvious advantages to studying healthy volunteers, particularly in the early phases of drug development, as healthy volunteers are more readily available and studies are not complicated by the necessity of stopping existing antipsychotic treatment. However, it is possible that patients may differ from healthy volunteers in their $EC_{50}$ as a result of the pathophysiology of the illness or secondary to prior antipsychotic treatment. Although it was not their primary interest, some researchers gave clues about the issue, reporting that patients and healthy volunteers show similar antipsychotic dose–occupancy relationship (Arakawa et al. 2010; Sparshatt et al. 2010). Nevertheless, the issue still remains to be answered.

In this study, we sought to determine if the antipsychotic concentration–occupancy relationship in patients with schizophrenia are equivalent to those in healthy volunteers. We measured the dopamine receptor occupancy induced by YKP1358, a novel atypical antipsychotic drug currently under clinical development, and compared $EC_{50}$ in patients and healthy volunteers. YKP1358 has in vitro affinities for $D_{2L}$, $D_{2S}$, and 5-$HT_{2A}$ receptors of 85, 91, and 0.83 nM ($K_i$ values), respectively.

## Methods

The present study was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, South Korea.

### Study design

This study was composed of two substudies—parts I and II—and was part of an ongoing evaluation of YKP1358. Part I was conducted in healthy volunteers, and part II was performed in patients with schizophrenia. The studies were designed to provide additional information on the PK and PD profile of YKP1358 and so showed design differences. However, both studies measured dopamine $D_2$ receptor occupancy of YKP1358 at a number of time points, allowing $EC_{50}$ to be calculated.

#### *Healthy volunteer study*

In part I, healthy volunteers followed a single oral dose and parallel group (100, 200, and 250 mg) study design (Fig. 1). The dopamine $D_2$ receptor occupancy was measured pre-dose, and at 2, 5, and 10 h after the YKP1358 administration. Serial blood samples were obtained for the measurement of YKP1358 plasma concentration. We have previously reported the relationship between plasma concentrations and dopamine $D_2$ receptor occupancy from the data obtained in part I (Lim et al. 2007). We found that the relationship between plasma concentrations and dopamine $D_2$ receptor occupancy was well predicted by a sigmoid $E_{max}$ model and that dopamine $D_2$ receptor occupancy by YKP1358

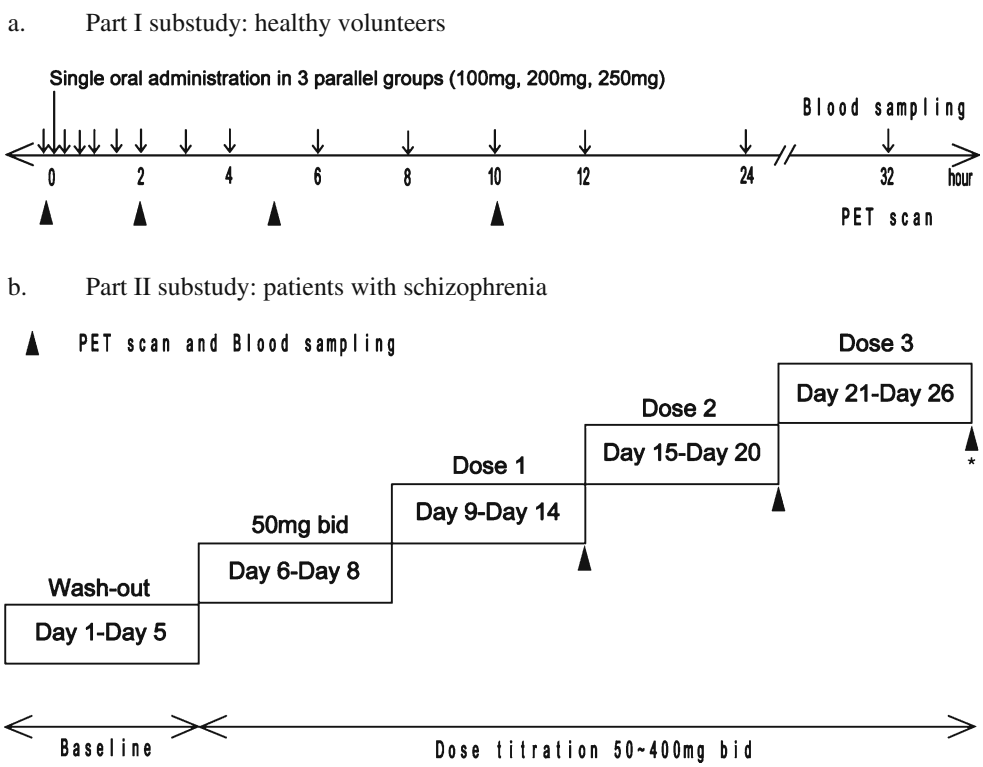



**Fig. 1** Diagram for study protocols in healthy volunteers (**a**) and patients with schizophrenia (**b**). *Asterisk* serial blood samples were obtained at 0 (pre-dose), 0.5, 1, 2, 3, 4, 8, and 12 h after the last administration

increased to 53–83% at 2 h and then decreased afterward, ranging from 40–64% at 5 h to 20–51% at 10 h.

*Patients with schizophrenia study*

Part II of the study was conducted according to a repeated oral dose titration, open-label study (Fig. 1). Previous antipsychotic medications were discontinued on day 1, which was followed by a washout period from day 1 to day 5±1. After the washout period, YKP1358 was administered orally twice per day for 18 to 24 days. All patients received 50 mg bid for the first 3 days; thereafter, the YKP1358 dosage was increased stepwise to the target range of 100–400 mg bid, according to the clinical response of each patient. After each escalation, the new dosage was maintained for 6±1 days. PET scans for the measurement of dopamine $D_2$ receptor occupancy were performed 12 h after the last administration of each step in the dose escalation. Blood samples for the measurement of YKP1358 were obtained just before the PET scan and at 0 (pre-dose), 0.5, 1, 2, 3, 4, 8, and 12 h after the last administration of the last step in the dose escalation.

Subjects

Subjects received a full explanation of the study and provided informed consent to participate.

*Healthy volunteers*

Subjects for part I were ten healthy non-smoking male volunteers. Screening tests for healthy volunteers included physical examination, vital signs, laboratory tests (hematology, blood chemistry, and urinalysis), and 12-lead electrocardiograms.

*Patients with schizophrenia*

The subjects for part II were eight patients with schizophrenia recruited from outpatient clinic. Men and women (aged 19 to 55 years) who met the DSM-IV criteria for schizophrenia were eligible for enrollment. For inclusion, the patients had to have experienced multiple episodes of schizophrenia and be able to provide informed consent and understand instructions in Korean. All patients were expected to be able to discontinue antipsychotic medications prior to the washout period.

*Exclusion criteria*

The exclusion criteria for all subjects were history or clinical evidence of significant respiratory, cardiovascular, renal, gastrointestinal, hepatic, endocrine, hematologic, neurologic (including ataxia), other chronic disease, or DSM-IV diagnosis (except schizophrenia in the patients); history of allergies, including drug allergies, except for

asymptomatic and seasonal allergies not requiring treatment during this study; clinically significant abnormalities in laboratory tests and the physical examination; any seizure disorders requiring anticonvulsant medication; clinically relevant ECG abnormalities; any psychiatric condition requiring concomitant psychotropic medication (except zolpidem or lorazepam treatment in the patients); child-bearing potential; a score greater than mild (score≥2) on any items of Abnormal Involuntary Movement Scale at screening or ongoing extrapyramidal symptoms requiring treatment with anticholinergic medication beyond the baseline; current use of psychoactive drugs confirmed by urine drug screening (barbiturate, cannabinoids, amphetamine/methamphetamine, opiates, and cocaine); or serious suicidal ideation.

All subjects were required to remain in the hospital for the duration of the study and abstain from caffeine or caffeine-containing products (e.g., coffee, cola, black tea, green tea, chocolate), grapefruit-containing products, and alcohol for the duration of hospitalization.

All participants were Koreans. Mean age (± SD), height, and body weight of healthy volunteers were 26.0±4.9 years, 174.7±6.4 cm, and 70.2±6.3 kg. All the patients were males with the average age (± SD) of 35.5±3.8, and the average body weight and height of the patients were 69.4±10.4 kg and 168.0±3.6 cm, respectively (Table 1).

## PET scanning procedure and image analysis

All PET data were obtained using the ECAT EXACT 47 scanner (Siemens-CTI, Knoxville, TN, USA). Dynamic 3D emission scans (15 s×8 frames, 30 s×16, 60 s×10, 240 s×10) were initiated concomitantly with a bolus injection of 370–740 mBq [$^{11}$C]raclopride and continued for 60 min. The acquired data were reconstructed in a 128×128×47 matrix with a pixel size of 2.1×2.1×3.4 mm by means of a filtered back-projection algorithm employing a Shepp-Logan filter, with a cutoff frequency of 0.3 cycles/pixel.

Static PET images obtained by combining all the frames of dynamic images were coregistered with the magnetic resonance (MR) images of the same individual. The MR images were used to define the region of interest (ROI) on the putamen and cerebellum (reference region; Ito et al. 1998). The ROI was transferred onto the dynamic PET images, to obtain the time–activity curves using the transformation parameters obtained by the coregistration of static PET and MR images.

A three-compartment model was employed for the kinetic analysis of the binding of [$^{11}$C]raclopride with the dopamine $D_2$ receptor (Ito et al. 1998). These compartments represent the concentration of radioligand in plasma ($C_p$), free or nonspecifically bound radioligand in brain ($C_f$), and specifically bound radioligand to receptors ($C_b$). The dopamine $D_2$ receptor binding potential (BP=$B_{max}/K_d$) in the putamen was calculated using a simplified reference tissue model in which the following equation was fit to obtain the BP and related parameters:

$$C_t(t) = R_1 C_r(t) + \left\{ k_2 - \frac{R_1 k_2}{(1+BP)} \right\} C_r(t) \otimes e^{\frac{-k_2 t}{(1+BP)}}$$

where $C_t(t)$ and $C_r(t)$ are the respective time–activity curves

**Table 1** Demographic characteristics of healthy volunteers and patients with schizophrenia

| | Healthy volunteers ($n$=10) | Patients with schizophrenia ($n$=8) | $p$ value[a] |
|---|---|---|---|
| Age (± SD) (year) | 26.0±4.9 | 35.5±3.8 | <0.001 |
| Sex (M/F) | 10/0 | 8/0 | |
| Height (± SD) (cm) | 174.7±6.4 | 168.0±3.6 | 0.016 |
| Weight (± SD) (kg) | 70.2±6.3 | 69.4±10.4 | 0.836 |
| Diagnosis ($n$) | | | |
| Paranoid | | 7 | |
| Residual | | 1 | |
| Previous antipsychotic medication ($n$) | | | |
| Risperidone | | 3 | |
| Olanzapine | | 1 | |
| Clozapine | | 2 | |
| Zotepine | | 1 | |
| Haloperidol | | 5 | |
| Chlorpromazine | | 2 | |
| Pimozide | | 1 | |
| Bromperidol | | 1 | |
| Clinical scales (± SD) | | | |
| PANSS | | 65.5±15.7 | |
| CGI-S | | 3.5±0.8 | |

[a] Independent $t$ test

for putamen and cerebellum, $R_1$ is the ratio of rate constants for ligand delivery from the plasma into the putamen and cerebellum, and $k_2$ is the rate constant for ligand washout from the putamen (Lammertsma and Hume 1996; Olsson and Farde 2001).

The dopamine $D_2$ receptor occupancy by YKP1358 was calculated as the percentage reduction of BP with drug treatment, compared with the baseline:

$$Occupancy(\%) = \frac{BP_{baseline} - BP_{drug}}{BP_{baseline}} \times 100$$

Baseline BPs in patients with schizophrenia

Though patients had a 5-day washout period, it was not long enough to remove antipsychotic drug from their brains because elimination half-life of antipsychotic drugs in the brain is longer than that in plasma (Tauscher et al. 2002). For this reason, we obtained the baseline BPs in the patients using an inhibitory $E_{max}$ model with individual serial BP data. We have assessed the reliability of the inhibitory $E_{max}$ model to calculate baseline $D_2$ levels in patients, finding that it shows good agreement with measured $D_2$ BP as evidenced by intraclass correlation coefficients greater than 0.8 (Kim et al. 2011):

$$BP = BP_{baseline} - \frac{I_{max} \times Conc^{r}}{IC_{50}^{r} + Conc^{r}}$$

where $I_{max}$ is the maximum inhibitory effect, Conc is plasma concentration of antipsychotic drug, $IC_{50}$ is the plasma concentration associated with 50% decrease of BP, and $\gamma$ is the Hill coefficient. In this model, we assumed that dopamine $D_2$ receptors would be totally occupied with YKP1358 when a supratherapeutic dose was administered and that the BP would be equal to zero. Under this assumption, $I_{max}$ was regarded as the baseline BP. Individual baseline BP was calculated from the individual binding potential data using nonlinear mixed effects modeling.

Nonlinear mixed effects modeling simultaneously estimates fixed effects and random effects in the inhibitory $E_{max}$ model. The fixed effects are parameters such as $I_{max}$, $IC_{50}$, and Hill coefficient which describe the relationship between the plasma drug concentration and BP in population. The random effects consist of inter-individual variability and residual variability. The inter-individual variability is between-subject variability of parameters which explain the difference between individual BP and population BP predicted from the model. The residual variability is within-subject variability or measurement error of BP which results in the difference between individual BPs from observation and prediction.

From the nonlinear mixed effect modeling, we obtained individual estimates of baseline BP as follows: Baseline $BP_i = I_{max} \cdot \exp(\eta_i \text{ of } I_{max})$,where Baseline $BP_i$ represents the true baseline BP value for the $i$th individual, $I_{max}$ is typical population value of the maximum inhibitory effect in the inhibitory $E_{max}$ model, and $\eta_i$ is inter-individual variability of the maximum inhibitory effect for $i$th individual. The calculation was performed using NONMEM ver. VI, level 1.1 software (GloboMax, Ellicott City, MD, USA).

Pharmacokinetic analysis

A non-compartmental analysis, using the WinNonLin® ver. 4.0.1 software (Pharsight, CA, USA), was employed to calculate the following pharmacokinetic parameters: area under the plasma concentration versus time curve (AUC) and elimination half-life ($t_{1/2}$). The actual measured values were taken for the maximum observed plasma concentration ($C_{max}$) and the time to reach the maximum observed plasma concentration ($T_{max}$). To compare the parameters in subjects with different dose, AUC and $C_{max}$ were corrected by dose.

Pharmacodynamic analysis

The relationship between plasma concentration of YKP1358 and dopamine $D_2$ receptor occupancy was analyzed with $E_{max}$ model as follows:

$$Occupancy(\%) = \frac{E_{max} \times Conc}{EC_{50} + Conc} \times 100$$

where $EC_{50}$ is the plasma concentration associated with 50% occupancy of dopamine receptors, $E_{max}$ is maximum occupancy, and Conc is plasma concentration. $E_{max}$ value was fixed to 100%. The analysis was performed using NONMEM ver. VI, level 1.1 software (GloboMax, Ellicott City, MD, USA).

Statistical analysis

According to the distribution of variables, non-parametric (Mann–Whitney test) tests were used to check for significant differences in PK and PD parameters between healthy volunteers and patients with schizophrenia.

## Results

Ten healthy male volunteers participated in part I substudy, and PET scans were performed in nine healthy volunteers because one of them rejected PET scan. Eight male patients took part in part II substudy. The average dosages of YKP1358 in each step were 50 mg (initial dose), 100 mg (dose 1), 177.5 mg (dose 2), and 247.5 mg (dose 3). Table 2

**Table 2** Doses escalated in each patient

| Subject ID | Initial dose | Dose 1 | Dose 2 | Dose 3 |
|---|---|---|---|---|
| YKP001 | 50 | 100 | 200 | 300 |
| YKP003 | 50 | 100 | 200 | 300 |
| YKP006 | 50 | 100 | 150 | 200 |
| YKP007 | 50 | 100 | 200 | 300 |
| YKP008 | 50 | 100 | 150 | 200 |
| YKP009 | 50 | 100 | 200 | 300 |
| YKP011 | 50 | 100 | 150 | 175 |
| YKP012 | 50 | 100 | 175 | 225 |
| Mean dose (mg bid) | 50 | 100 | 177.5 | 247.5 |

shows the individual dosages. Among the eight patients, two patients (YKP011 and YKP012) were additionally scanned 2 h after the last administration of the last step. One patient (YKP009) did not complete the serial blood sampling for pharmacokinetic analysis after the last administration of the last step in the dose escalation.

### Pharmacokinetic parameters

The mean $T_{max}$ (± SD) was 0.6±0.1 h in healthy volunteers and 0.7±0.3 h in patients. The $C_{max}$/dose (± SD) was 0.9±0.7 ng/ml/mg in healthy volunteers and 0.6±0.3 ng/ml/mg in patients. The AUC/dose from time 0 to 12 h was 1.0±0.4 ng h/ml/mg in healthy volunteers and 1.0±0.3 ng h/ml/mg in patients. The $T_{max}$, $C_{max}$/dose, and the AUC/dose were not different between healthy volunteers and patients ($T_{max}$: $U$=32.0, $p$=0.813; $C_{max}$/dose: $U$=29.0, $p$=0.601; AUC/dose: $U$=28.0, $p$=0.536). However, healthy volunteers showed longer elimination half-life (± SD) than patients (healthy volunteers 7.1±1.7 h, patients 4.3±1.3 h; $U$=29.0, $p$=0.005; Table 3).

### Pharmacodynamic parameters

The inhibitory $E_{max}$ model for the estimation of baseline BP in patients provided a good fit for the relationship between binding potential and plasma concentration (Fig. 2). The mean baseline BPs (± SD) were 2.5±0.4 in healthy volunteers and 3.1±0.4 in patients. The baseline BPs differed between patients and healthy volunteers ($U$=9.5, $p$=0.008).

The relationship between plasma concentration and dopamine $D_2$ receptor occupancy showed a good fit to the sigmoid $E_{max}$ model (Fig. 3). The $EC_{50}$ was 7.6 ng/ml (95% CI 6.2–9.0) in healthy volunteers and 8.6 ng/ml (95% CI 7.4–9.9) in patients ($U$=17.0, $p$=0.142).

## Discussion

We investigated the dopamine $D_2$ receptor occupancy of YKP1358 to compare the $EC_{50}$ in healthy volunteers and patients with schizophrenia. The $EC_{50}$ reflects the relationship between plasma concentration and dopamine receptor occupancy. Our finding that the $EC_{50}$ did not differ between healthy volunteers and patients with schizophrenia indicates that healthy volunteers can be used to predict the dopamine receptor occupancy induced by YKP1358 in patients. Moreover, it supports the use of PET imaging in the evaluation of other dopamine receptor antagonist drugs.

However, we did find a difference in baseline BP between healthy volunteers and patients. The difference in age between the two groups can cause the different baseline BP. The age-related decline of dopamine $D_2$ receptor density has been well documented (Inoue et al. 2001; Wong et al. 1997). The patients with schizophrenia were significantly older than the healthy volunteers (Table 1). However, the different baseline BP does not seem to result from the difference in age between the two groups because the patients who were older than the healthy volunteers showed higher baseline BP. Even if difference in age had an effect on baseline BP, the age-related change in dopamine $D_2$ receptor is reported not to influence the concentration–occupancy relationship (Uchida et al. 2009). For this reason, it would be more reasonable that the difference in baseline BP resulted from the previous exposure to antipsychotic drugs. It has been reported that exposure to antipsychotic drugs may induce an upregulation of dopa-

**Table 3** Pharmacokinetic parameters (± SD)

| | Healthy volunteers ($n$=10) | Patients with schizophrenia ($n$=7) | $p$ value[a] |
|---|---|---|---|
| $T_{max}$ (h) | 0.6±0.1 | 0.7±0.3 | 0.813 |
| $C_{max}$/dose (ng/ml/mg) | 0.9±0.7 | 0.6±0.3 | 0.536 |
| $AUC_{12}$/dose (ng h/ml/mg) | 1.0±0.4 | 1.0±0.3 | 0.601 |
| $t_{1/2}$ (h) | 7.1±1.7 | 4.3±1.3 | 0.005 |

$T_{max}$ the time to reach the maximum observed plasma concentration, $C_{max}$ the maximum observed plasma concentration, $AUC_{12}$ the area under the plasma concentration versus time curve from time zero to 12 h, $t_{1/2}$ the elimination half-life

[a] Mann–Whitney $U$ test

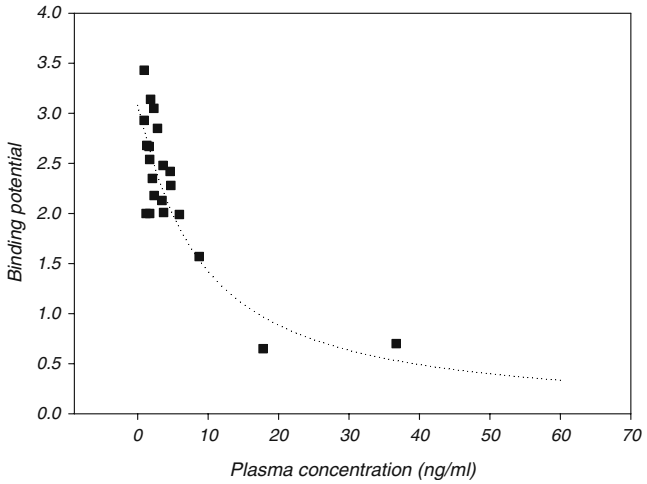


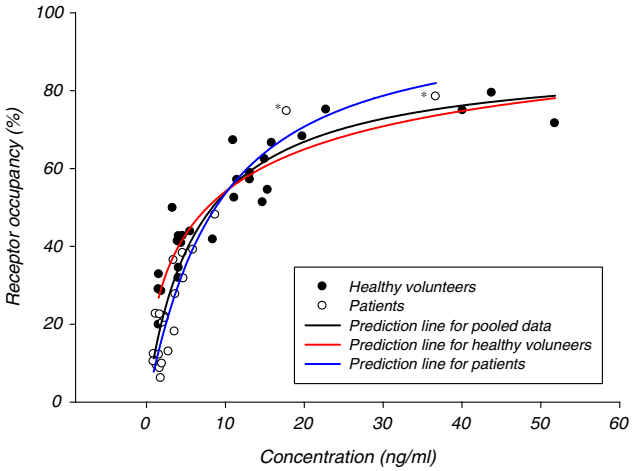

**Fig. 2** The relationship between binding potential and plasma concentration in terms of inhibitory $E_{max}$ model after the administration of YKP1358 in patients with schizophrenia

mine receptors. This was first described in rats (Burt et al. 1977) and subsequently observed in the postmortem brains of patients with schizophrenia (Lee et al. 1978; Mackay et al. 1982). The latter postmortem studies could not exclude the possibility that an elevation in dopamine $D_2$ receptors is intrinsic to schizophrenia, but subsequent molecular imaging studies in drug-naïve patients indicate that any elevation is small at most (Laruelle 1998). In vivo neuroimaging study also revealed that dopamine $D_2$ receptor binding was increased after long-term exposure to antipsychotic drugs (Silvestri et al. 2000). The range of dopamine receptor upregulation was 20% to 25% in rat brain and around 34% in human brain (Burt et al. 1977; Silvestri et al. 2000). We also observed that the baseline BP in patients was 24% higher than that in healthy volunteers, which is in agreement with the previous results. This upregulation might lead to difference in dopamine $D_2$ receptor profile between patients treated with antipsychotic drugs and healthy volunteers.

However, the dopamine receptor upregulation did not affect the relationship between plasma concentration and receptor occupancy in terms of $EC_{50}$ (Fig. 3). This is in accordance with the previous report by Ginovart et al. (2009). They reported that though constant infusion of haloperidol led to a robust upregulation of striatal $D_2$ receptors in cats, the dopamine receptor occupancy measured by [$^{11}$C]raclopride PET remained stable. In addition, no significant change in the receptor affinity for the antagonist was observed. Assuming that the distribution of drug from the plasma to the brain and its affinity for dopamine receptor are not influenced by previous antipsychotic medications, the observation is explainable because the ratio of drug-bound receptors ($B$) to drug-unbound receptors ($U$) should be unchanged under the assumption, and the receptor occupancy can be defined as follows: $\{1/(1+U/B)\}\times 100$. Together with the report by Ginovart et al. (2009), our data indicate that dopamine $D_2$ receptor occupancy as a pharmacodynamic parameter useful for new antipsychotic drug development seems not to be influenced by the upregulation.

**Fig. 3** The relationship between the dopamine $D_2$ receptor occupancy of YKP1358 and its plasma concentration in terms of sigmoid $E_{max}$ model (**a**) and estimated parameters (**b**). *Asterisk* the dopamine receptor occupancies in patients were measured 12 h after the last administration in each step of dose escalation, but YKP011 and YKP012 were additionally scanned 2 h after the last administration of the last step

a. Receptor occupancy vs Concentration




b. $E_{max}$ model parameters

| Parameters | Healthy volunteers | Patients with schizophrenia | Pooled data |
|---|---|---|---|
| **$E_{max}$(%)** | 100 | 100 | 100 |
| **$EC_{50}$(ng/ml) (95%CI)** | 7.6 (6.2-9.0) | 8.6 (7.4-9.9) | 8.0(6.8-9.2) |

We compared the $EC_{50}$ between healthy volunteers and patients with schizophrenia. The $EC_{50}$ itself is estimated with drug concentration and dopamine receptor occupancy. We measured plasma concentration and dopamine receptor occupancy in both healthy volunteers and patients with schizophrenia and calculated the $EC_{50}$ from the data. However, the measurements for healthy volunteers were conducted after single administration of YKP1358 and those for patients with schizophrenia were performed at trough level in steady state. According to the study design, the patients with schizophrenia were longer exposed to YKP1358 than the healthy volunteers. It could lead to upregulation of dopamine receptors in the patients. Nonetheless, as discussed earlier, the change in status of dopamine receptor by exposure to antipsychotic drugs might not affect the concentration–occupancy relationship (Ginovart et al. 2009), and it means the possible upregulation of dopamine receptors in the patients might not have any effect on our conclusion.

However, the difference in time points at which the measurements were obtained could have affected our results. There is evidence that the time courses of plasma concentration and dopamine receptor occupancy are dissociated (Tauscher et al. 2002). It indicates that the relationship between drug concentration and receptor occupancy can be changed according to the time points when they are measured. The dissociation may come from the difference in drug concentration between the plasma and the effect site in the brain (Pleuvry 2008). In the case of YKP1358, it was previously reported that the equilibrium half-life of the effect site in the brain with the plasma was as short as 20 min (Lim et al. 2007). It suggests that the difference in drug concentration between the plasma and the effect site in the brain could be small and that the difference in the time points when the data were obtained between the subject groups could have small effect on our conclusion. However, it is still required to confirm our results with data acquired from the similar study protocols.

## Conclusion

We found no evidence that $EC_{50}$ was different between patient and control groups. This supports the use of healthy volunteers in the evaluation of dopamine receptor blocking drugs.

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