Serotonin transporter occupancy of SKL10406 in humans: comparison of pharmacokinetic-pharmacodynamic modeling methods for estimation of occupancy parameters

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SKL10406, triple monoamine reuptake inhibitor, is a novel antidepressant candidate. A PET study was performed to investigate the occupancies of serotonin and dopamine transporters (SERT and DAT) in human brain, and the relationship between SKL10406 concentration and SERT occupancy was assessed using pharmacokinetic-pharmacodynamic (PK-PD) modeling methods. Fifteen healthy volunteers were given SKL10406 100 mg/day for 6 days or 150 mg/day for 6 days after 100 mg/day for 4 days. Each subject underwent full PK sampling for SKL10406 and PET scans at pre-dose, 4 h and 16 h after dosing at a steady state to investigate the occupancies of SERT and DAT using $^{11}$C-DASB and $^{11}$C-PE2I, respectively. Naive pooled method (NPM) and nonlinear mixed-effect methods (ME) including a direct ME (DME) and an effect compartmental ME (EME) were used (NONMEM Ver. 7.2). Six and five subjects completed the studies for SERT and DAT, respectively. The final estimates of $E_{\text{max}}$ (53.4%) and $E_{50}$ (11.8 ng/mL) from DME were relatively lower than those from NPM ($E_{\text{max}}$, 74.1%; $E_{50}$, 36.8 ng/mL) and EME ($E_{\text{max}}$, 68.6%; $E_{50}$, 40.2 ng/mL). DAT occupancy results were not modeled because of lower occupancies. The results showed that the dosage regimens may be applied in patient studies. However, difference between estimation methods alerts that ME may not be a recommendable analysis tool for sparsely sampled PET scan data.

Introduction

Recently, triple monoamine reuptake inhibitors (TRI) have gained attention as candidates for the next generation of antidepressants. One advantage of TRI is their ability to elevate synaptic dopamine levels, thereby improving hedonic responses by modifying the function of both the nucleus accumbens and dorsal putamen, with the latter responsible for increased motor speed.

In addition, a useful concept of TRI is that stimulatory dopaminergic activity added to serotonin norepinephrine reuptake inhibitors (SNRI) may compensate for deleterious effects on sexual function caused by chronic serotonin transporter inhibition whilst maintaining antidepressant efficacy. This possibility is inferred from reports suggesting that dysfunction of dopaminergic reward mechanisms is related to anhedonia, a major symptom of depression,[1] and dopamine mediates sexual behavior and ejaculation through different dopamine receptor sub-types.[2] Furthermore, the idea of using dopaminergic agonists for selective serotonin reuptake inhibitors (SSRI)-induced sexual dysfunction[3] has been exemplified in a report that SSRI-treated patients exhibit improved sexual function after addition of bupropion, a norepinephrine/dopamine reuptake inhibitor.[4]
SKL10406 is a small molecule under clinical development as a potential TRI with IC_{50} values of 19.5, 9.5, and 10.9 nM for reuptake inhibition of human serotonin transporter (SERT), norepinephrine transporter (NET), and dopamine transporter (DAT), respectively.[5] Based on these profiles, a positron-emission tomography (PET) imaging study for SKL10406 was performed in healthy subjects to assess SERT and DAT occupancy as a proof of concept of its pharmacological activity in the human brain. PET imaging technology is currently used in drug development as a non-invasive method to determine the distribution and target binding potential of drug candidates during early clinical development.[6,7]

Several reports have asserted the superiority of nonlinear mixed effect pharmacokinetic-pharmacodynamic (PK-PD) modeling (ME) of occupancy data obtained from human PET studies compared to the simple, conventional naïve pooled method (NPM) that does not consider interindividual differences of the maximum effect (E_{\text{max}}) or plasma concentration that achieves 50% of the maximum effect (EC_{50}).[8–10] Thus, both NPM and ME were carried out to determine SERT occupancy data for SKL10406. As for ME, both direct ME (DME, using individual predicted plasma drug concentration) and effect compartmental ME (EME), which assumes the existence of an effect compartment, were performed.

This study attempted to interpret the implications of different E_{\text{max}} and EC_{50} estimates from NPM and ME, and to discuss several of their respective caveats with respect to interpretation of PET study data using ME. Although ME is an essential tool for drug development and enables the understanding of drug action in a quantitative manner, it is sometimes misunderstood as a tool for deriving complicated models from sparsely sampled clinical trial data. Rather, the use of ME is maximized when the number of subjects and observations per subjects are large enough to give reliable parameter estimates. The assumption of an effect compartment in ME for sparsely sampled PET study data raises additional concerns because it is impossible to observe any delay in peak occupancy behind peak drug concentration during real PET studies, where only two points of occupancy are measured per subject.

In support of the occupancy delay and thus the use of effect compartment modeling, Kim et al. exemplified the decay of the occupancy slower (longer half-life) than that of drug concentration.[8] However, the slower decay of the occupancy (effect) is a commonly observed finding when EC_{50} is much lower than observed drug concentrations regardless of the time delay. Berges et al. compared ME with NPM and the two-stage method (TSM) for analysis of simulated PET study data, and showed that ME is better than TSM in terms of estimating interindividual variability (IVI) of EC_{50} values.[9] However, these results do not appear to be reliable evidence for advocating the use of ME, because TSM is useful only when the number of observation per subject is sufficiently large to estimate reliable PD parameters for each individual. Consistent with this concept, there have been no PET studies analyzed by TSM. Moreover, Berges et al. found that NPM works as well as ME in terms of bias and precision.[9] Lastly, Abanades et al. reported that occupancy resulting from repeated doses is better predicted by ME devised using single dose results than by a simple E_{\text{max}} model in a study with 10 subjects.[10] To the best of our knowledge, this is the only example to demonstrate the marginal superiority of ME (p-value = 0.0488).

Can ME provide robust estimates of E_{\text{max}} and EC_{50} from a PET study with only two points of PD markers (occupancy) per subject? While it may not be possible to obtain a simple ‘yes’ or ‘no’ answer to this question, as well as to determine whether DME or EME are preferable, it was nevertheless tried to compare the pros and cons of these different methods. Specifically, estimates from two models (DME and EME) were evaluated for the analysis of SERT occupancy in healthy subjects and the necessity of a more critical attitude towards sophisticated PK/PD analysis tools such as ME was proposed.

Methods

Subjects

Three subjects were enrolled for each of 4 cohorts. All subjects were healthy volunteers who met the following inclusion criteria: age 18–50 years; body mass index between 19.0 and 30.0 kg/m²; body weight less than 125.0 kg; physical dimensions appropriate for fitting into the MRI scanner; negative pregnancy test, and use of acceptable birth control methods throughout the study and for a period of 30 days following the completion of the study in the case of female volunteers. Key exclusion criteria were as follows: use of medication suspected of affecting SERT or DAT binding within 1 week prior to the first dosing; history of serious medical illness (i.e. renal or hepatic insufficiency) or psychiatric illness; history of significant alcohol or substance abuse, or cigarette smoking.

The study protocol was approved by the Institutional Review Board of the study center (Kendle Early Stage, Toronto, ON, Canada) and the Research Ethics Board of the PET Centre (Centre for Addiction and Mental Health, Toronto, ON, Canada) in accordance with Good Clinical Practices. All applicable regulatory requirements were met. Written informed consent was obtained from all volunteers after the study procedures were explained in full.

Study Design

This was an open-label and multiple-oral dose study conducted in healthy volunteers. Subjects allocated to Cohorts 1 and 2 received 100 mg SKL10406 daily (50 mg every 12 h for 6 days. Subjects in Cohorts 3 and 4 received 100 mg SKL10406 daily (50 mg every 12 h) for 4 days followed by 150 mg SKL10406 daily (75 mg every 12 h) for the next 6 days. Each dose was administered with approximately 240 mL of water.

All subjects underwent a baseline (pre-dose) PET scan prior
to the start of treatment. Subjects then received multiple daily doses of SKL10406 to achieve steady state plasma levels of the study drug, at which time subjects received two post-dose PET scans: one estimated to coincide with the maximum plasma concentration (C\text{max}) approximately 4 hours post-dose on the last day of dosing and the other to coincide with estimated plasma concentration at the end of the dosing interval (C\text{ trough}), approximately 16 hours after the final dose. For Cohorts 1 and 2, PET scans were obtained 4 hours after the first dose on Day 6 and 16 hours after the last dose on Day 7. For Cohorts 3 and 4, PET scans were obtained 4 hours after the first dose on Day 10 and 16 hours after the last dose on Day 11.

Subjects assigned to Cohorts 1 and 3 underwent radiotracer \([\text{I}^\text{11}}\text{C}]\text{ DASB}\) PET scans to measure SERT occupancy, whereas those assigned to Cohorts 2 and 4 underwent radiotracer \([\text{I}^\text{11}}\text{C}]\text{ PE2I}\) PET scans to measure DAT occupancy. Because subjects were scanned before and after treatment with the study drug, each subject served as their own control; no placebo or other comparators were utilized in this study.

Peripheral venous plasma concentrations of SKL10406 were measured prior to the first dose on Day 1, at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 16 hours after the first dose on Day 6 (cohorts 1 and 2) or Day 10 (cohorts 3 and 4).

**Image Acquisition and Analysis**

For subjects in Cohorts 1 and 3, 370 MBq of \([\text{I}^\text{11}}\text{C}]\text{ DASB}\) was intravenously injected before PET scans. For subjects in Cohorts 2 and 4, 370 MBq of \([\text{I}^\text{11}}\text{C}]\text{ PE2I}\) was injected instead of \([\text{I}^\text{11}}\text{C}]\text{ DASB}\). \([\text{I}^\text{11}}\text{C}]\text{ DASB}\) and \([\text{I}^\text{11}}\text{C}]\text{ PE2I}\) were synthesized as described elsewhere.[11–15] Images were obtained in 15 one-minute frames followed by 15 five-minute frames.

T\text{T}_1-weighted and proton density brain MRI scans were obtained for each subject and co-registered to respective PET scans using a mutual information algorithm. PET images were obtained using a whole body PET camera system, Siemens Biograph HiRez XVI (Siemens Molecular Imaging, Knoxville, TN, USA), which had both a CT and a PET component. The CT component of the system was used both for subject positioning as well as for acquiring the transmission scan, which was converted into an attenuation correction for the PET scan. Prior to scanning, subjects were fitted with a thermoplastic head fixation mask (Orfit Industries, Wijnegem, Belgium) to prevent significant head movement during scan acquisition.

Nondisplaceable binding potential (BP\text{ND}) was measured, and is hereafter referred to as binding potential (BP). In images obtained for measuring SERT occupancy, the bilateral striatum was chosen as the primary region of interest for analysis because \([\text{I}^\text{11}}\text{C}]\text{ DASB}\) uptake is high and homogenous in the striatum[11], and absolute test-retest differences in SERT binding potential for this region are low (<10%).[12,13] To measure binding potential in regions of interest, the Logan non-invasive method[16] implemented with a standard software package (PMOD Technologies Ltd, Zurich, Switzerland) was used. For \([\text{I}^\text{11}}\text{C}]\text{ PE2I}\) PET, the primary region of interest was whole striatum. The kinetics of \([\text{I}^\text{11}}\text{C}]\text{ PE2I}\) have been described as a two tissue compartment model[17] and the Logan analysis without blood sampling was suitable for measuring BP in radioligands with this property for which a reference tissue is present.[16]

**Data Analysis**

Transporter occupancy (TO) was viewed as the percentage of target sites blocked by treatment. The occupancy for regions of specific binding was defined as follows:

\[
\text{TO (\%)} = \frac{\text{BP}_{\text{baseline}} - \text{BP}_{\text{treatment}}}{\text{BP}_{\text{baseline}}} \times 100 \tag{1}
\]

where BP refers to binding potential. PK and PD data for SKL10406 were analyzed using NONMEM (version 7.2, Icon Development Solutions, Ellicott City, MD, USA) with the G77 FORTRAN compiler. For PD data, as shown in Figure 3, DAT occupancy data was not sufficient to estimate maximum occupancy at the doses used in our study because an occupancy greater than 50% was observed only twice. Thus, a PD model for DAT occupancy could not be established. For SERT occupancy data, the simple conventional concentration-effect model (NPM), the direct PK-PD model (simple E\text{MME} model using mixed-effect analysis, DME), and the effect compartmental PK-PD model (EME) were compared.

**Population PK Model Building for ME**

A first-order conditional estimation method with interaction was used, and the concentration-time course of SKL10406 was investigated comparing one- and two-compartment\textsuperscript{\textendash}order elimination models with and without absorption lag time. The IIIV of each parameter was estimated using an exponential error model. Additive, proportional, and combined error models were tested for residual errors. Structural model selection was based on diagnostic scatter plots and the likelihood ratio test in which the change in objective function value approximated the \(\chi^2\) distribution (\(\chi^2_{0.05}>3.84\)). In the case of non-nested models, the value of Akaike information criteria was used.

**NPM**

The $\text{PRED}$ in NONMEM and the additive error model were employed for analysis. A simple $\text{E}_{\text{max}}$ model was used to explore the relationship between the observed plasma concentration and striatal SERT occupancy without estimating IIIV (omega fixed to be 0) for the $\text{E}_{\text{max}}$ or $\text{EC}_{\text{50}}$. It was assumed that TO was instantaneously influenced by drug concentration without time delay, resulting in hysteresis. The $\text{E}_{\text{max}}$ model equation for TO was as follows:

\[
\text{TO (\%)} = \frac{\text{BP}_{\text{baseline}} - \text{BP}_{\text{treatment}}}{\text{BP}_{\text{baseline}}} \times 100 \tag{1}
\]
where $E_{\text{max}}$ is the maximum effect of TO, $EC_{50}$ is the plasma concentration that achieves 50% of the maximum effect, and $C_p$ is the observed plasma concentration of SKL10406.

**DME**

Unlike NPM, which uses the observed plasma concentrations of SKL10406, DME and EME were conducted using predicted concentrations obtained from the population PK step. Individual PK parameters were fixed to those obtained from the PK modeling process (sequential approach) because the TO data was quite sparse compared with PK data. DME used a simple $E_{\text{max}}$ model without any time delay between PK and TO, which was identical to those used for NPM analysis. IIV was estimated for $E_{\text{max}}$ and $EC_{50}$.

**EME**

EME was also conducted based upon the sequential approach used in DME. Because CNS drugs exert their pharmacological effects by binding to targets in the brain after passing the blood-brain barrier, it is assumed that changes to PD markers (TO in this study) are always preceded by changes in plasma concentration. To reflect this time delay between PK and TO, an effect compartmental model was employed (Fig. 2).

The differential equation for the effect compartment drug concentration was:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C - C_e)$$

where $k_{e0}$ is the equilibrium rate constant and $C$ and $C_e$ are the drug concentrations in the central and effect compartment, respectively. The $E_{\text{max}}$ equation was also used, but $C_e$ was substituted for the observed or individual predicted drug concentrations.

IIV was successfully estimated for $EC_{50}$, but not for $E_{\text{max}}$, because the estimation process was terminated. A proportional error model was used to describe the residual variability because successful convergence was not achieved with either the additive or combined error models for both DME and EME. The criteria of model selection for PD model were identical to those used for the PK modeling step.

**Model Evaluation**

Bootstrapping and visual predictive checks (VPCs) were conducted to evaluate the final PK and PD models. One thousand bootstrap-resampled datasets were estimated to find the 95% confidence interval (CI) of each parameter using the final PK and PD models. In addition, VPCs were performed by overlaying observations with the 12.5th, 50th, and 87.5th percentile curves of 1,000 simulated data sets from the PK and PD models.

**Results**

Fifteen healthy volunteers participated in this study, four of which discontinued participation during the study because of scheduling problems (n=2) and adverse events (n=2). Thus, a total of 11 subjects who fulfilled both the PK and PD studies (six for SERT and five for DAT) were included in the final analysis (Table 1).}

**SERT and DAT Occupancies**

Six healthy subjects completed all measurements for SERT

![Untreated condition](image1)

![Treatment condition](image2)

Figure 1. Occupancy effect of SKL10406 upon [11C] DASB (top) and [11C] PE2i (bottom) brain uptake

![SKL10406 oral intake](image3)

![Figure 2. Effect compartmental PK-PD model (EME) for SKL10406. C, drug concentration in the central compartment; $C_e$, drug concentration in the effect compartment; CL, clearance; $k_{s0}$, absorption rate constant; $k_{e0}$, equilibrium rate constant; Q, inter-compartmental clearance](image4)
occupancy with \(^{11}\text{C}\) DASB PET in Cohorts 1 and 3, one of which refused PET scanning on Day 11. The median striatal occupancy values for Cohort 1 at 4 and 16 hours were 40% and 17%, respectively, while those for Cohort 3 were 53% and 15%, respectively (Table 2). DAT occupancy data was obtained from five subjects. The median striatal occupancy values for Cohort 2 at 4 and 16 hours were 25% and 27%, respectively, while those for Cohort 4 were 54% and 29%, respectively (Table 2).

With respect to DAT occupancy, \(E_{\text{C50}}\) alone was tried to be estimated after fixing \(E_{\text{max}}\) value at 100% because the distribution of concentration and occupancy data did not appear to be feasible for estimation of robust \(E_{\text{max}}\) value as shown in Figure 3B. However, the resulting estimate (162 ng/mL) was greater than most of the observed concentrations shown in Figure 3B, indicating that the estimate was not very reliable. For these reasons, DAT occupancy was not modeled.

### Population PK Model

The population PK of SKL10406 in the 11 subjects was best described by a two-compartment first-order absorption and elimination model.

### Table 1. Subject demographics

<table>
<thead>
<tr>
<th></th>
<th>SKL10406 100 mg (Cohorts 1, 2)</th>
<th>SKL10406 150 mg (Cohorts 3, 4)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects (male/female)</td>
<td>6 (6/0)</td>
<td>5 (4/1)</td>
<td>11 (10/1)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.5 ± 4.51</td>
<td>44.8 ± 11.82</td>
<td>43.5 ± 8.21</td>
</tr>
<tr>
<td>Race (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Black or African American</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>26.4 ± 2.07</td>
<td>24.8 ± 3.73</td>
<td>25.7 ± 2.90</td>
</tr>
</tbody>
</table>

Demographics are presented for the population who completed all measurements including PET procedures. Continuous variables are described as the mean ± standard deviation.

### Table 2. Transporter occupancy according to dosage groups and sampling time

<table>
<thead>
<tr>
<th>Transporter type</th>
<th>Dosing schedule</th>
<th>Sampling time</th>
<th>Occupancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg bid</td>
<td>at 4 hr</td>
<td>40 (40-68)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at 16 hr</td>
<td>17 (13-62)</td>
<td></td>
</tr>
<tr>
<td>75 mg bid</td>
<td>at 4 hr</td>
<td>53 (31-64)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at 16 hr</td>
<td>15 (6-25)</td>
<td></td>
</tr>
<tr>
<td>DAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg bid</td>
<td>at 4 hr</td>
<td>25 (13-26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at 16 hr</td>
<td>27 (3-35)</td>
<td></td>
</tr>
<tr>
<td>75 mg bid</td>
<td>at 4 hr</td>
<td>54 (33-75)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at 16 hr</td>
<td>29 (4-54)</td>
<td></td>
</tr>
</tbody>
</table>

Occupancy (%) is presented as the median (range).

### Table 3. Final model estimates of population pharmacokinetic and pharmacodynamic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical value</th>
<th>IIV (%)(^a)</th>
<th>RSE (%)(^b)</th>
<th>Bootstrap median (95% CI)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pharmacokinetic parameters (ME)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>49.6</td>
<td>62.2</td>
<td>19.0</td>
<td>43.7 (32.9-68.9)</td>
</tr>
<tr>
<td>(V_2) (L)</td>
<td>176</td>
<td>41.6</td>
<td>14.4</td>
<td>169 (15.9-221)</td>
</tr>
<tr>
<td>(k_a) (h(^{-1}))</td>
<td>5.05</td>
<td>57.6</td>
<td>18.6</td>
<td>16.6 (5.01-32.4)</td>
</tr>
<tr>
<td>(V_1) (L)</td>
<td>63.7</td>
<td>33.2</td>
<td>18.3</td>
<td>51.2 (38.8-133)</td>
</tr>
<tr>
<td>Q (L/h)</td>
<td>21.1</td>
<td>18.3</td>
<td>19.0</td>
<td>51.2 (15.0-32.8)</td>
</tr>
<tr>
<td>ALAG (h)</td>
<td>0.336</td>
<td>27.0</td>
<td>0.429</td>
<td>0.429 (0.18-0.48)</td>
</tr>
<tr>
<td><strong>Pharmacodynamic parameters (NPM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_{\text{max}}) (%)</td>
<td>74.1</td>
<td>7.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_{\text{C50}}) (ng/mL)</td>
<td>36.8</td>
<td>31.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pharmacodynamic parameters (DME)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_{\text{max}}) (%)</td>
<td>53.4</td>
<td>20.7</td>
<td>8.31</td>
<td>53.1 (44.2-65.9)</td>
</tr>
<tr>
<td>(E_{\text{C50}}) (ng/mL)</td>
<td>11.8</td>
<td>44.9</td>
<td>23.6</td>
<td>12.1 (7.23-28.5)</td>
</tr>
<tr>
<td><strong>Pharmacodynamic parameters (EME)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_{\text{max}}) (%)</td>
<td>68.6</td>
<td>4.62</td>
<td>69.9</td>
<td>69.9 (50.3-104.8)</td>
</tr>
<tr>
<td>(E_{\text{C50}}) (ng/mL)</td>
<td>40.2</td>
<td>68.3</td>
<td>34.3</td>
<td>44.2 (12.9-140)</td>
</tr>
<tr>
<td>(K_{e0}) (h(^{-1}))</td>
<td>0.288</td>
<td>9.65</td>
<td>0.286</td>
<td>0.286 (0.202-0.409)</td>
</tr>
</tbody>
</table>

CL, clearance; \(V_2\), volume of central compartment; \(k_a\), absorption rate constant; \(V_1\), volume of peripheral compartment; Q, inter-compartmental clearance; ALAG, absorption lag time; \(E_{\text{max}}\), maximum effect; \(E_{\text{C50}}\), concentration that achieves 50% of maximum effect; \(K_{e0}\), equilibrium rate constant.

\(^a\)Inter-individual variability, \(^b\)Relative standard error of parameter estimates was obtained from the NONMEM covariance step. \(^c\)95% confidence intervals (CI) were estimated by applying the final pharmacokinetic or pharmacodynamic model to 1,000 re-sampled data sets.
elimination model with combined residual error (Fig. 2). The inclusion of absorption lag time decreased OFV (△OFV = 7.95) and improved the individual concentration plots. In the final model, the estimates of clearance (CL), volume of the central compartment, and absorption rate constant were 49.6 L/h, 176 L, and 5.05 h⁻¹. The IIV (coefficient of variation, CV%) of these parameters were 62.2%, 41.6%, and 0% (not estimated), respectively. The population PK parameter estimates and their bootstrap results (median and 95% confidence interval) are summarized in Table 3. Although one of the subjects was suspected to be a poor metabolizer of SKL10406 based on his drug concentration profile, which was much higher than the other study subjects (Figure 4, observation of suspect PM in PK graph), his PK data was included in the PK and PD analysis steps without exception.

PD Parameter Estimates for SERT Occupancy

Figure 3 shows the relationship between observed plasma concentration and striatal SERT occupancy in the six subjects of Cohorts 1 and 3. The final parameter estimates for NPM, DME, and EME for striatal SERT occupancy are summarized in Table 3. The estimates of $E_{\text{max}}$ (53.4%) and $EC_{50}$ (11.8 ng/mL) of DME were relatively lower than those of NPM and EME.

The equilibrium half-life calculated from the equilibrium rate constant ($k_{e0} = 0.288$ h⁻¹) in EME was 2.41 hours. Results of the VPC are shown in Figure 4. In Figure 4, Vertical dashed lines are used to show the delay in peaks between PK and occupancy for EME.

Safety

Among the fifteen subjects who received at least one study dose, 10 subjects experienced at least one treatment emergent adverse event. The most common adverse events were insomnia followed by nausea, headache, dizziness, tachycardia, tachyphrenia, constipation, dry mouth, diarrhea, and back pain. Most subjects had normal vital signs, ECG results, and physical examination findings. No subject showed any suicidal ideation or behavior as assessed by the Columbia Suicide Severity Rating Scale.

Discussion

The relationship between plasma concentration and SERT occupancy of SKL10406 was explored in this study using three different models, namely, NPM, DME, and EME. The values of $E_{\text{max}}$ (53.4%) and $EC_{50}$ (11.8 ng/mL) estimated from DME were substantially different despite using the same IIV terms. The use of the effect compartment model in EME provided clues for the observed difference. Because the $EC_{50}$ in EME was the effect compartment concentration ($C_{e}$) for which the time-course was behind
Figure 4. Visual predictive check plots of the final pharmacokinetic and pharmacodynamic models. 1,000 data sets were simulated using the parameter estimates of the final model. Prediction interval curves of the 12.5th, 50th, and 87.5th percentiles from 1,000 simulated datasets from the PK and PD models were overlaid with observed data. Vertical dashed lines indicate the $T_{\text{max}}$ of SKL10406 and are shown to demonstrate a delay in predicted occupancy changes by EME compared with DME.
that of the plasma concentration, its estimate might be substantially greater than the EC50 in DME. Thus, PD parameter estimates of SKL10406 varied substantially between the different PK-PD modeling methods, indicating that care should be taken when interpreting them; e.g., the number of data points and the existence of influential subjects.

To date, ME has been used as a standard method to find reliable parameter population estimates and their distribution (IIV) for almost of PK and PD studies; however, its use in occupancy studies for development of antidepressants is still relatively new. Some researchers have asserted that ME is superior to conventional NPM for the analysis of PET study results,[8, 9] similar to PK-PD studies in the other therapeutic areas. The usefulness of ME is greatest when IIV and residual errors are well discernible by the support of raw data. In other words, the number of observed PD endpoints (occupancy) per subject should be larger than the number of PD parameters (for example, two when estimating both Emax and EC50). Similarly, although there is no absolute cut-off point, the number of subjects should also be large. However, occupancy is measured only twice (excluding baseline measurement) per subject, and there are only 10-12 subjects in a typical PET study due to the high cost and difficulty associated with the measurement procedure. Importantly, in this study, data from as few as six subjects gave robust PD estimates from ME. However, it should be noted that data from an influential subject (in our case, a subject with overly high drug concentrations) might alone determine the value of PD parameter estimates (Emax in our case) if NPM is used. For this reason, it was asked whether ME could help to avoid this shortcoming of NPM. Unfortunately, the answer differed according to the model that was used, as shown in our results. In the case of DME, IIV was successfully estimated for Emax in the PD, where the influential subject’s higher occupancy was reflected as a positive value of IIV instead of inflating the estimate of the population Emax. EME, which has been advocated by some researchers,[8, 10] was not able to identify a IIV term for Emax (the estimation step did not converge with IV on Emax) and thus the population Emax value was similar to that from NPM (Table 3) in our study. As mentioned in the method section, EME assumes the existence of an effect compartment for estimating the delayed time-course of the effect in comparison with drug concentration changes. Thus, compared with DME, EME utilizes an additional parameter (k0) for its estimates. Addition of only one more parameter may terminate the estimation process by NONMEM, especially when the information (numbers of subjects and observed PD markers) is not sufficient, which is often the case in PET studies. In all of the reports that recommended ME over NPM,[8-10] Emax is fixed to 100% without IIV and EC50 is the only estimated parameter. Despite the use of such simplified estimation conditions, the usefulness of ME over NPM was marginal in only one of the three reports[10] described in the introduction section in this report. The limitations of ME in PET studies exemplified in our SERT occupancy data in six subjects were not expected to disappear even if the number of subjects increased to between 10 or 12, the typical number of subjects used for PET studies, because the computational burden imposed by simultaneous estimation of two parameters (Emax and EC50) as well as the influence of outliers is rooted in the sparseness of occupancy measurements rather than the number of subjects.

Most SSRI such as paroxetine exhibit approximately 80% SERT occupancy in the human brain at clinically effective doses. [12,13,18] However, multiple monoamine transporter reuptake inhibitors or multimodal neurotransmitter enhancers may be efficacious at lower SERT occupancy than needed for SSRI, presumably because of simultaneous inhibition of NET or DAT which may also contribute to their antidepressant effects. For example, milnacipran, one of SNRI, exhibits approximately 40% SERT occupancy at its typical clinical dosage regimen in depressive patients. [19] Lu AA21004, which acts as both a SERT inhibitor and 5-hydroxytryptamine receptor antagonist/agonist, exhibits a SERT occupancy of approximately 50% at its effective dosage regimen in a Phase II clinical trial.[20] Likewise, the SERT occupancy of SKL10406 as a potential TRI may be targeted lower than that of typical SSRI. Specifically, 50 mg bid and 75 mg bid multiple dosing of SKL10406 resulted in approximately 30–50% median SERT occupancy in the simulation step based upon the final PK-PD models used in this study. Thus, when viewed from the standpoint of SERT occupancy, these dosage regimens appear to be suitable for later phase clinical trials in patients.

For DAT occupancy, the optimal range for achieving an antidepressant effect is unknown; however, higher dopamine levels raise safety concerns such as reinforcing effects, psychosis and abuse liability. For example, bupropion has 14% DAT occupancy at clinically effective doses, while methylphenidate, a controlled substance due to its abuse potential, is reported to block more than 50% of DAT at its clinical doses.[22] Although PD model for DAT occupancy was not established, it was assumed that desirable DAT occupancy levels of SKL10406 are greater than 14% and less than 50% based on these results, and thus 50 mg bid may be better than 75 mg bid because one of the subjects in the 75 mg bid group (Cohort 4) exhibited a DAT occupancy higher than 50% (75% at 4 h after dosing).

The relationship between SKL10406 concentration and SERT occupancy was estimated in this study by three different methods, and occupancy ranges were well predicted from the estimated occupancy parameters. Although ME that can estimate IIV and covariate in population PK/PD modeling is a sophisticated method, the simple NPM may be better for estimating both of the occupancy parameters (Emax and EC50, herein) with sparsely sampled PET data in a small number of subjects as in the present study.

Overall, substantial occupancy of SERT and DAT in human brain was demonstrated by repeated dosing of SKL10406 50 mg
bid or 75 mg bid and the SKL10406 concentration and SERT occupancy relationships found in the models suggest that the dosage regimens may be used for clinical trials in patients.

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Conflict of interest
Jung-Shin Park is an employee of SK Biopharmaceuticals Co. Ltd. All the other authors declare no conflict of interest.

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