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Original Article

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Physiologically-based pharmacokinetic model for clozapine in Korean patients with schizophrenia

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ABSTRACT

Clozapine has been used as a treatment of schizophrenia. Despite its large interindividual variability, few reports addressed the physiologically-based pharmacokinetic modeling and simulation (PBPK M&S) of clozapine in patients. This study aimed to develop a PBPK M&S of clozapine in Korean patients with schizophrenia. PBPK modeling for clozapine was constructed using a population-based PBPK platform, the SimCYP® Simulator (V19; Certara, Sheffield, UK). The PBPK model was developed by optimizing the physiological parameters of the built-in population and compound libraries in the SimCYP® Simulator. The model verification was performed with the predicted/observed ratio for pharmacokinetic parameters and visual predictive checks (VPCs) plot. Simulations were performed to predict toxicities according to dosing regimens. From published data, 230 virtual trials were simulated for each dosing regimen. The predicted/observed ratio for the area under the curve and peak plasma concentration was calculated to be from 0.78 to 1.34. The observation profiles were within the 5th and 95th percentile range with no serious model misspecification through the VPC plot. A significant impact on age and gender was found for clozapine clearance. The simulation results suggested that 150 mg twice a day and 150 mg three times a day of clozapine have toxicity concerns. In conclusion, a PBPK model was developed and reasonable parameters were made from the data of Korean patients with schizophrenia. The provided model might be used to predict the pharmacokinetics of clozapine and assist dose adjustment in clinical settings.

Keywords: Clozapine; PBPK; Korean; Schizophrenia Patients

INTRODUCTION

Clozapine is an atypical antipsychotic drug that belongs to the tricyclic dibenzodiazepine group [1,2]. This drug is generally used to the treatment of schizophrenia, especially for patients who are intolerant or refractory to the side effects of typical antipsychotic drugs [2,3]. Clozapine has a lower risk of undesired neurological effects and may ameliorate some negative symptoms compared to other antipsychotic drugs [1,3]. However, large interindividual variations in plasma clozapine levels at the same dose have been reported [4,5]. The starting dose is 12.5 mg once or twice a day. The total daily dose can be increased

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Conflict of Interest

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by 25–50 mg per day, if well-tolerated, to achieve a target dose of 300–450 mg per day by the end of two weeks [6,7]. Clozapine presents a narrow therapeutic window and Therapeutic drug monitoring (TDM) is recommended [1,3,8]. Serum concentrations of clozapine < 250 and > 750 ng/mL are associated with relapse and increased risk of intoxication, respectively [8]. Most articles suggested that concentrations > 600–1,000 ng/mL were more likely to evoke adverse effects, especially seizure activity [9]. In addition, the treatment should only be used in specific situations, such as signs of toxicity, inadequate clinical response, onset of seizures, concomitant liver disease, concurrent use of smoking or caffeine, changes in concurrent medications, and suspected noncompliance [3].

Clozapine is quickly and almost completely absorbed after oral administration with time to maximum plasma concentration (T_{max}) of 1.5–2 h in which the maximum effect of the drug appears approximately 4 h after administration [1,10]. However, its bioavailability is only about 27%–50% due to the first-pass metabolism. In addition, approximately 95% of the drug is bound to plasma proteins [1,10]. Clozapine is principally eliminated by extensive hepatic metabolism that generates two main metabolites of *N*-desmethylclozapine (norclozapine) and clozapine *N*-oxide [1,8]. The metabolism includes several cytochrome P450 (CYP) isoenzymes, CYP1A2 in particular, and enzymes 3A4, 2C19, and 2D6 [1,3,8]. *N*-desmethylclozapine is an active metabolite capable of affecting the dopamine D2 and D3 receptors, serotonin receptors, histamine receptors, and muscarinic M1 [1,3,8].

Physiologically-based pharmacokinetic modeling and simulation (PBPK M&S) is a tool that can be applied to assess the pharmacokinetic (PK) profile of a drug based on its preclinical absorption, distribution, metabolism, and excretion data. It can be employed to estimate the exposure in a target organ or tissue after drug administration, taking into account the rate of absorption, metabolism and disposition in the organ [11]. On the other hand, it can also be used to assess the impacts of various physiological parameters such as gender, age, ethnicity, or disease status on pharmacokinetics, as well as dose regimen and drug-drug interaction [12]. The use of this tool currently are remarkable at whole stages of the drug development process [12]. In addition, research on PBPK modeling of clozapine is rare in Korean patients with schizophrenia.

This study aims to develop a platform and predict for various clinical situations through PBPK M&S of clozapine in Koreans. In particular, the psychiatric drug model, which is not easy for clinical research on patients, is thought to be very useful to supplement clinical research.

METHODS

Clinical study design

Enrolled in this study are 23 patients with schizophrenia aged 20–60 years (42.26 ± 8.54, mean ± standard deviation) with 13 and 10 males and females, respectively. Patients who were administered clozapine for three months prior to participation in the study without changes in the dose were enrolled. They were regularly given clozapine to control their symptoms. Patients who received other antipsychotics for the purpose of treating schizophrenia were excluded. All subjects voluntarily gave written informed consent prior to entering the study. Each subject was physically healthy as indicated by physical examinations, their medical histories, and standard clinical laboratory tests. Exclusion criteria included those who had a medical history of kidney disease, liver disease, cardiovascular diseases, gastrointestinal

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disorders, hepatitis, drug abuse, blood disorders and alcoholism, HIV sero-positive or AIDS. Subjects with an allergy to clozapine or atypical antipsychotics drugs (e.g., benzodiazepines) or atypical antipsychotics were excluded. The study was conducted following the guidelines of Good Clinical Practice and the Declaration of Helsinki [13]. The study protocol was approved by the institutional review board of the six hospital sites (Catholic University of Korea, Yeouido St. Mary's Hospital, Konkuk University Chungju Hospital, Naju National Hospital, National Center for Mental Health, Dongguk University Medical Center, and Wonkwang University Hospital).

All subjects received 100 mg of Clozaril Tab. (Novartis) twice daily after breakfast (8:00 AM) and dinner (8:00 PM) for 10 days. The subjects were subsequently orally given one tablet of 100 mg clozapine with 240 mL of water. Blood samples via IV catheter were collected at −72, −48, and −24 h before administration; 0 h (pre-dose); and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, and 12 h after the last drug administration. Furthermore, blood samples were centrifuged and plasma was separated, transferred to new tubes, and stored at −70°C until assay.

Determination of clozapine in plasma

The whole blood collected from the subjects was immediately placed in the heparin tube, centrifuged, and frozen at below −70°C until analysis. After thawing the frozen plasma samples at room temperature, 50 μL of the plasma was taken and 50 μL of atomoxetine (1 μg/mL) was added as an internal standard substance. In addition, 1,000 μL of 100% acetonitrile was added and centrifuged for 5 min at 12,000 rpm after 10 s of vortexing. Transferred to a clean tube was 100 μL of the supernatant. Afterward, 1,000 μL of 100% acetonitrile was added for dilution. Furthermore, 200 μL of the dilution solution was transferred to the vial and 5 μL was injected into the liquid chromatography–tandem mass spectrometry. Analyses were carried out on Agilent 6490 Triple Quad LC/MS (Agilent Technologies, Santa Clara, CA, USA). The separation of analytes was performed on Unison UK C18 (2.0 mm × 50 mm, 3 μm; Imtakt Co., Kyoto, Japan) using an isocratic elution of 10 mM ammonium formate and acetonitrile (40:60, *v*/*v*) at 0.3 mL/min. The mass transitions (*m*/*z*) used were 327.1→270.1 and 256.2→44.1 for clozapine and atomoxetine, respectively. Pharmacokinetic parameters were calculated by the noncompartmental method using Phoenix™ WinNolin® (Pharsight Corp., Mountain View, CA, USA).

Development of PBPK model for clozapine

The whole-body PBPK model was developed using a SimCYP® simulator version 19 (Certara, Sheffield, UK). The input parameters for clozapine in the PBPK model are presented in **Table 1**. The SimCYP® software compound library was used except for f_a which was optimized through sensitivity analysis. To optimize the absorption model, the optimized fraction absorbed from the dosage form (f_a) was obtained through a sensitivity analysis. Sensitivity analyses were performed as followed: the lower and upper bounds were set to 0.1 and 1, respectively. The uniform-step approach (total ten steps) was applied to obtain the optimized value. The optimized results were assessed by comparing the ratio of C_{max} and area under the curve (AUC) (predicted/observed).

Model verification

The PK of clozapine in multiple-dose patients with schizophrenia was predicted. The set up model was verified with observed clinical data from previously reported literatures [10,14,15]. Virtual subjects were matched to the study volunteers for similar age range, dosing regimen, and ethnicity. The data for built-in population libraries used for the verification were as

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Table 1. Physicochemical properties and pharmacokinetic parameters of the clozapine used for the development of the PBPK model

Parameters	Input value	Reference
Physicochemical* properties		Default
Molecular weight (g/mol)	326.800	
Log *p*	3.500	
Compound type	Small molecule	
pKa (monoprotic base)	7.750	
Blood-to-plasma partition ratio	0.845	
Fraction unbound in plasma	0.055	
Absorption		
Absorption model	First-order	
f_a	0.600	Optimized by sensitivity analysis
k_a (h^{-1})	2.220	Default
fu_{gut}	1.000	Default
Q_{gut} (1 h^{-1})	16.787	SimCYP predicted†
P_{eff}, man (10^{-4} cm s^{-1})	6.862	SimCYP predicted†
Distribution		
Distribution model	Minimal PBPK model	
V_{ss} (L/kg)	1.600	Default
Elimination		
Clearance type	Enzyme kinetics	
N-demethylation		
CYP1A2 V_{max} (pmol/min/pmol)/K_m (μM)	13.1/14.2	Default
CYP2C9 V_{max} (pmol/min/pmol)/K_m (μM)	2.58/12.0	
CYP2C19 V_{max} (pmol/min/pmol)/K_m (μM)	7.68/9.45	
CYP2D6 V_{max} (pmol/min/pmol)/K_m (μM)	4.57/19.5	
CYP3A4 V_{max} (pmol/min/pmol)/K_m (μM)	13.5/222	
N-oxidation		
CYP1A2 V_{max} (pmol/min/pmol)/K_m (μM)	4.94/8.99	Default
CYP3A4 V_{max} (pmol/min/pmol)/K_m (μM)	11.6/91.6	

f_a, fraction absorbed from the dosage form; fu_{gut}, fraction unbound in the gut; k_a, first-order absorption rate constant; K_m, the maximum rate; P_{eff}, *man* effective permeability in man; Q_{gut}, gut blood flow; V_{max}, the maximum rate; V_{ss}, volume of distribution at steady state.
*Physicochemical data were obtained from the SimCYP® library; †These parameters were predicted using previously validated function in SimCYP®.

follows; "V15R1_KoreanHealthy_Population_SeoulNationalUniversityAndHospital" for healthy Koreans of the current study, "Sim-Korean Healthy Volunteers" for healthy Thai of Tassaneeyakul et al. study [10] and "Sim-Healthy Volunteers" for healthy Caucasians of both Golden et al. and Srameck et al. studies [14,15].

Ten virtual trials with 23 subjects were conducted for each clinical study. PK parameters, including area under the curve over the last dosing ($AUC_{last,ss}$), clearance (CL), maximum plasma concentration at steady-state ($C_{max,ss}$), and time at which $C_{max,ss}$ was observed T_{max} were assessed.

The predictive performance using the PBPK models was determined using a VPC. The median and its 5th–95th percentile confidence intervals of simulated plasma concentration–time profiles were plotted along with observed data for a visual predictive check (VPC) plot.

Age, gender and weight variables were used as covariate evaluation variables for clozapine clearance. The simulation for covariate evaluation was performed in 1,000 subjects (100 trials with 10 subjects, aged between 20 and 60 years) using the virtual population libraries included in the simulator. Demographic information of the patients used in the development of the model is presented in **Table 2**.

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Table 2. Characteristics of the patients in the clinical study (n = 23)

Characteristics	Female (n = 10)	Male (n = 13)
Age (yr)	40.3 ± 9.2	43.8 ± 7.3
Body weight (kg)	62.8 ± 6.7	69.4 ± 9.3
Height (cm)	158.1 ± 3.8	170.8 ± 5.4
Serum creatinine (μmol/L)	74.5 ± 11.2	81.7 ± 12.8
Human serum albumin (g/L)	4.4 ± 0.1	4.4 ± 0.3
Hematocrit (%)	40.3 ± 2.4	42.2 ± 4.0

Values are mean ± standard deviation.
Human serum albumin was calculated from the data of 7 females and 11 males.

The mean differences between the two groups were compared using the *t*-test. A p value < 0.05 was considered significant. Analyses were performed using SPSS version 25 (IBM, New York, NY, USA).

Based on the usual dose of 100–200 mg/day, a simulated dose of 100 mg once a day and 100 mg twice a day (total 200 mg/d) of clozapine were administered for two weeks [6,9]. Based on the FDA-approved target dose of 300–450 mg/day for clozapine, a simulated dose of 150 mg twice a day (total 300 mg/d), and 150 mg three times a day (total 450 mg/d) administered for two weeks were simulated [6,7,9]. The simulation results are shown in **Fig. 1**. The simulations were conducted with 10 virtual trials with 100 subjects in each clinical study.

RESULTS

The input parameters for clozapine in the PBPK model are presented in **Table 1**. Except f_a in absorption model, all the input parameters were obtained from SimCYP® default library. The first-order absorption and minimal PBPK model were applied. Effective permeability in man ($P_{eff,man}$) were estimated using the first-order absorption parameters (absorption constant, fraction absorbed from the dosage form). For the elimination profile for clozapine, the intrinsic clearances by recombinant CYPs were applied. Metabolic pathways of clozapine were set to N-demethylation and N-oxidation. CYP2C9, 2C19, and 2D6 were set to contribute to N-demethylation, and CYP1A2 and CYP3A4 were set to contribute to both N-demethylation and N-oxidation.

The performance of the simulations was assessed by the ratio of the mean predicted and observed PK parameters following the administration of 100 mg clozapine twice daily as shown in **Table 3**. The mean of each predicted PK parameters was compared with the published mean in the corresponding clinical study, and the ratio of the predicted/observed values was obtained. The model was considered to fit well if the ratio of the predicted/observed values were within 30% (0.7–1.3) [16]. Data for healthy Korean and Caucasian populations were obtained from the SimCYP® equipped population library. The predicted and observed ratios were included in the range 0.78–1.34 (0.7–1.3, 30% range of the arithmetic mean ratio). The ratio of the AUC_{SS} (1.34) in Tassaneeyakul et al. [10] overlays the boundary line, but the model was accepted if applied to the 0.5–2.0 range in other literature [17,18].

Fig. 2 shows that the clozapine model appropriately described the clozapine pharmacokinetic profile following 100 mg of multiple oral administrations in patients with schizophrenia. The observation profiles were within the 5th and 95th percentile range with no serious model misspecification.

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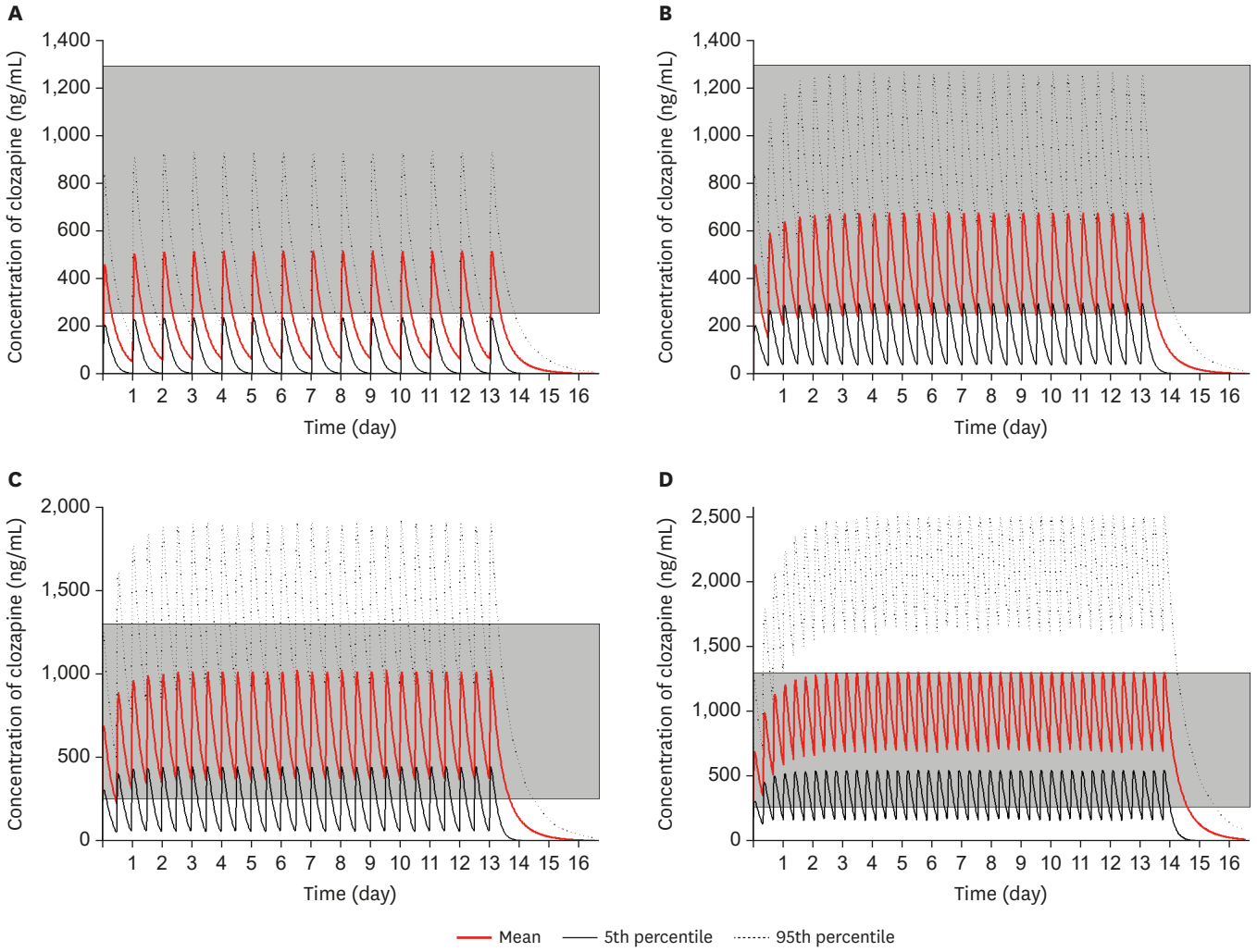

Figure 1. Simulated plasma clozapine concentration after (A) 100 mg once daily (B) 100 mg twice a day (C) 150 mg twice a day and (D) 150 mg three times a day clozapine administrations for 2 weeks (n = 1,000). Red lines indicate mean values. Solid and dashed lines indicate a 5th percentiles and 95th percentiles, respectively. Gray areas represent the therapeutic concentration range (250–1,300 ng/mL).

A significant age and gender effect were found for clozapine clearance. The weight was not significant with *p*-value 0.188. The effects of age and gender on drug exposure ($AUC_{last,ss}$, $C_{max,ss}$) were evaluated in the simulation results. In **Table 4**, we found that the $C_{max,ss}$ and $AUC_{last,ss}$ ratios between two groups by age and gender were within the range of 0.88–1.14. Virtual subjects in the subgroup aged 20–40 years had a significantly increased clozapine clearance compared with the subjects aged 41–60 years (25.98 versus 22.89 L/h, *p*-value 0.001). Also, females had a significantly lower mean clearance compared with males (23.40 versus 25.49 L/h, *p*-value 0.021). Although there were statistical differences, the differences of absolute means were relatively small by age groups or gender groups. These are insufficient evidences to determine dose-adjustment between each group.

The plasma concentration–time profiles for clozapine following oral administration of 100 mg once daily, 100 mg twice a day (total 200 mg/d), 150 mg twice a day (total 300 mg/d) and 150 mg three times a day (total 450 mg/d) clozapine administrations for 2 weeks are shown in **Fig. 1**. As a result of the simulation, the mean C_{max} values of 100 mg once daily and twice a

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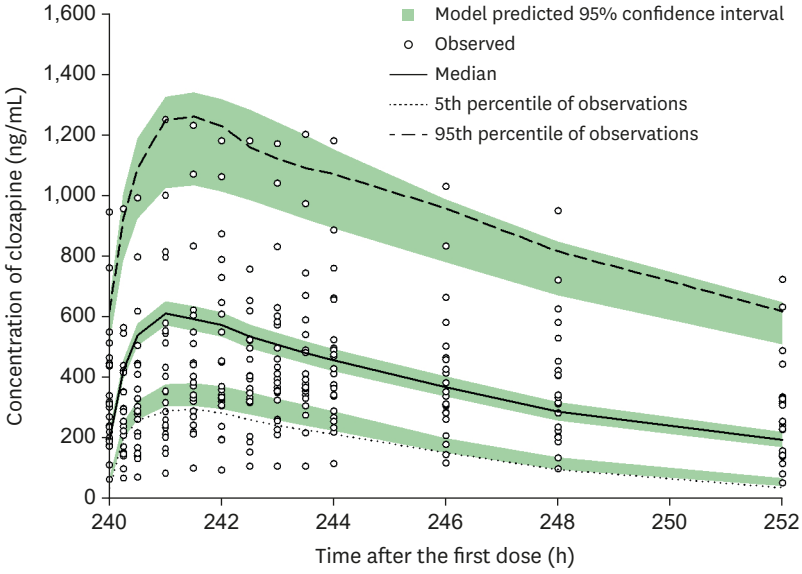

Figure 2. Overlay of observed (circles) plasma concentration and simulated (lines) steady-state plasma concentration–time profile of clozapine. Black lines indicate the overall mean for the virtual populations. The dotted and dashed lines indicate the 5th and 95th percentile confidence intervals, respectively. Area indicates the model predicted 95% confidence interval for the median, 5th, 95th percentile.

Table 3. Mean predicted (pred) and observed (obs) pharmacokinetic parameters following administration of clozapine 100 mg twice a day

Study reference	$AUC_{last,ss}$ (ng/mL)	CL (L/h)	C_{max} (ng/mL)	$T_{max,ss}$ (h)
Current study*				
Observed†	4,641	-	541.3	2.74
Predicted‡	5,339	24.72	676.6	1.34
Pred/obs ratio	1.15	-	1.25	-
Tassaneeyakul et al. [10]				
Observed†	3,994	31.75	551.3	1.97
Predicted‡	5,337	24.73	676.4	1.34
Pred/obs ratio	1.34	0.78	1.23	-
Golden and Honigfeld [14]				
Observed†	3,443	-	455.5	2
Predicted§	3,121	43.17	448.9	1.28
Pred/obs ratio	0.91	-	0.99	-
Sramek et al. [15]				
Observed†	2,781	-	358	1.93
Predicted§	3,121	43.17	448.9	1.28
Pred/obs ratio	1.12	-	1.25	-

$AUC_{last,ss}$, area under the curve over the last dosing; $AUC_{0-12h,ss}$, area under the plasma concentration–time curve from the last dosing at steady state; C_{max}, maximum concentration; CL, clearance; $T_{max\ ss}$, time to maximum plasma concentration at steady state.
*Non-compartmental analysis; †patients with schizophrenia; ‡healthy Koreans; §Caucasian.

Table 4. Assessment of $C_{max,ss}$ and $AUC_{last,ss}$ by age and gender

Variables	C_{max} (ng/mL)	$AUC_{last,ss}$ (ng/mL)
Age (yr)		
20–40	654.2 (287.3)	5,062.1 (2,715.9)
41–60	719.0 (314.3)	5,728.1 (3,003.3)
Ratio	0.91	0.88
Gender		
Female	731.1 (312.2)	5,640.4 (3,068.2)
Male	641.7 (286.3)	5,147.7 (2,659.5)
Ratio	1.14	1.10

$C_{max,ss}$, maximum plasma concentration at steady-state; $AUC_{last,ss}$, area under the curve over the last dosing.

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day dosing regimen were approximately 500 and 650 ng/mL, respectively. The mean trough concentrations of 100 mg once daily and twice a day dosing were approximately 80 and 250 ng/mL, respectively. Mean C_{max} values of 150 mg twice a day and 150 mg three times a day dosing regimen were approximately 1000 ng/mL and 1300 ng/mL, respectively. Mean trough concentrations of 150 mg twice a day and 150 mg three times a day dosing regimen were approximately 400 ng/mL and 700 ng/mL, respectively. The mean C_{max} were shown to be above 1,000 ng/mL for healthy adults (**Fig. 1C and D**). Based on the upper therapeutic margin of 1,300 ng/mL [19], the percent concentrations which exceeded 1,300 ng/mL during the 2-weeks administrations were calculated from each simulation. Approximately 0.6% subjects in 100 mg once daily dosing, 4.8% subjects in 100 mg twice a day dosing, 21.7% in 150 mg twice a day dosing, and 42.9% subjects in 150 mg three times a day dosing had the C_{max} values exceeded 1300 ng/mL. Based on the subtherapeutic margin of 250 ng/mL [19], the percentage of concentrations less than 250 ng/mL which reached a steady state (3–13 days) during the 2-week administrations were calculated for each simulation. Approximately 97.9% of subjects with 100 mg once daily dosing, 61.0% of subjects with 100 mg twice a day dosing, 42.2% of subjects with 150 mg twice a day dosing, and 13.2% of subjects with 150 mg three times a day dosing achieved C_{min} values less than 250 ng/mL.

DISCUSSION

This study developed the PBPK model for clozapine in Korean patients with schizophrenia. The clozapine plasma concentration–time profiles were well predicted without misspecification though the VPC plot. The ratios of the predicted and observed pharmacokinetic parameters were included in the acceptable ranges.

It is believed that only one published study exists constructing the PBPK modeling of the clozapine in patients [20]. The first-order absorption model for clozapine produced the best fit in this study. The first-order absorption model and the advanced, dissolution, absorption, and metabolism (ADAM) model were performed for comparison. The selection of the optimum model depended on how well the plasma concentration–time profile is predicted. In comparison to the ADAM model, the first-order absorption model improves simulation performance. In the Ghoneim et al. [20], observed data was from patient with renal impairment or hepatic impairment whereas patients in the current clinical study did not have renal impairment or hepatic impairment. Difference of physiological characteristics might have contributed to determination of the model structure.

In the Ghoneim et al. [20] reported that the ADAM model produced the best fit in young male adults with renal and hepatic impairments. The simulation performance of the ADAM model was superior to the first-order absorption by applying physiological factors that significantly affect absorption. One possible explanation for such a discrepancy may be the different underlying diseases for populations.

Significant intra- and interindividual pharmacokinetic variabilities for clozapine were observed in routine TDM [2,21,22]. The influences of age, gender, smoking status, CYP1A2 activity, and other factors on plasma clozapine concentrations have been previously reported [2,5,23,24]. However, the results of previous studies on these factors are inconsistent [2,23,25]. This study investigated the impacts of age and gender on clozapine clearances. It was found to be statistically significant with clozapine concentrations being higher in

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patients aged between 41 and 60 years than in patients aged between 20 and 40 years. In addition, Ghoneim et al. [20] reported that aging is accompanied by decreased in renal and hepatic functions and changes in plasma protein concentrations. These results are in agreement with those of other studies [25-29]. However, Tang et al. [23] found no statistically significant relationship between age and plasma clozapine concentrations, which is consistent with Pagelo et al. [30] and Perry et al. [31]. Although the discrepancies of these studies have not yet been revealed, the generalization of the studies is questionable due to its small sample size and limited age range [25]. Additional data is required for this area. In the present study, an increase in clozapine clearance was observed in men (about 2.09 L/h higher) compared with women. It is consistent with the gender effect reported by Cooper et al. [32] and Centorrino et al. [33]. It is assumed that females could have lower CYP1A2 activity and eventually higher clozapine concentrations and lower clearance [19,25].

Clozapine has been used as an atypical antipsychotic for schizophrenia [9,34]. However, the range of safe therapeutic doses for clozapine has not been established due to the large variations in individual responses [9,35]. In many studies, the lower limit of clozapine levels considered effective for most patients is in the range of 200–550 ng/mL, with most reports preferring the levels of at least 350–420 ng/mL [9,35,36]. Some papers have noted that clozapine concentrations range from 1,000 to 1,300 ng/mL for the upper limit at which clinical advantages are no longer obtained or side effects no longer occurred [34]. Clozapine toxicity causes several side effects including coma, seizures, low consciousness, delirium, tachycardia, hypotension, cardiac arrest, arrhythmias, aspiration, and respiratory depression [9,37]. It was possible to confirm that it was out of the recommended therapeutic plasma level and the risk of adverse reactions was predicted. This is thought to be caused by wide variations in metabolic enzyme activity that are affected by genetic factors, patients' characteristics (age, gender, and weight), cigarette status, concomitant medications, and other environmental factors [9]. Therefore, this suggested that clozapine levels are more clinically reliable than the recommended range of dosage when determining safe and efficient clozapine doses for individual patients [9].

This study is valuable because the clinical results of patient data for psychiatric drugs are very difficult to obtain. Therefore, the current study provides useful information about PBPK M&S for clozapine in patients with schizophrenia. However, this study has several limitations. First, smoking status was not assessed. In the clinical study, subjects were not allowed smoking during the study. Smoking status has previously been identified as a statistically significant covariate affecting clozapine clearance [2,23,24,38]. Clozapine clearance is likely affected by smoking status because the CYP1A2 activity is greater in smokers than in nonsmokers. It has been reported that smoking can increase clozapine clearance [2]. Second, the biological measures of CYP1A2 activity and norclozapine are lacking. The CYP1A2 is the major CYP isoform in clozapine metabolism and CYP1A2 activity is a significant factor in determining clozapine dose [20]. The primary metabolite of clinical significance is *N*-desmethylclozapine (norclozapine), which has demonstrated an affinity for D2, 5-HT1C, and 5-HT2 receptors [15]. Dailly et al. [5] suggested that considerable variation in clozapine plasma levels may be related to clozapine hepatic metabolism. Therefore, adding information about CYP1A2 activity and norclozapine will make more accurate and improved predictions for individual clozapine clearance. Third, a large study including patients from other ethnicities is needed to confirm the results of this study. Finally, obesity and metabolic syndrome are not considered. Although obesity and metabolic syndrome were more prevalent in patients with schizophrenia [39], the mean (range) body mass index (BMI) of the

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subjects in the clinical study was 24.4 kg/m^2 (19.7–29.7 kg/m^2) and subjects who have other serious disease were excluded the study. Because of the characteristics of data used for model building, the influence of other comorbid disease was not considered enough. Moreover, the BMI values (ranges: 18.2–30.6 [10], 16.8–41.3 [14,15]) of virtual subjects used in the model evaluation showed a statistical difference with those in the clinical study. The difference in BMI between the groups could affect the validity of the model.

In summary, the developed PBPK model involving Korean patients with schizophrenia adequately characterized the PK profiles of clozapine. The results of this study showed that age and gender were significant covariate variables for clozapine clearance. These results can be useful tools to predict the PK of clozapine and support dose refinement other related decision-making in clinical fields.

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