Population Pharmacokinetic Analysis of Daikenchuto, a Traditional Japanese Medicine (Kampo) in Japanese and US Health Volunteers

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ABSTRACT

We constructed population pharmacokinetic (PK) models for the five constituents of daikenchuto (DKT), a traditional Japanese herbal medicine. Data were collected from two randomized PK studies conducted in Japan and the United States. Participants received single oral doses of 2.5 g, 5 g, and 10 g of DKT. The plasma concentrations of five DKT constituents—hydroxy-α-sanshoool (HAS), hydroxy-β-sanshoool (HBS), 6-shogaool (6S), 10-shogaool (10S), and ginsenoside Rb1 (GRB1)—were determined by liquid chromatography-tandem mass spectrometry. A total of 1859 samples from 55 participants (US, n = 36; Japanese, n = 19) were included in the analysis. Population PK models of HAS, HBS, 6S, and 10S were best described by a one or two-compartment model with a bolus input. On the other hand, the model of GRB1 was best described by a one-compartment model with nonlinear extravascular input. Among the covariates evaluated, body mass index (BMI) and age were found to influence oral clearance (CL/F) and volume of distribution (Vd/F) for HAS and HBS, respectively. The influence of body weight on CL/F and Vd/F for 6S was demonstrated. Marked differences were observed in mean plasma concentrations of HAS and HBS between Japanese and US participants. However, the simulation results indicated that the difference in plasma concentrations may be attributed to the difference in demographic factors such as BMI, body weight, and age, whereas ethnic difference between the Japanese and US participants was considered minimal.

Introduction

Daikenchuto (DKT) is a traditional Japanese herbal medicine that consists of extracted three botanical raw materials: Japanese pepper, processed ginger, and ginseng radix (Kono et al., 2009). Since its approval as a prescription drug in 1986 by the Japanese Ministry of Health, Labor and Welfare, DKT has been widely used by gastroenterologists and surgeons for the treatment of various gastrointestinal disorders, such as postoperative ileus and obstructive bowel disease (Itoh et al., 2002; Ohya et al., 2003; Kono et al., 2009). Consequently, the gastrointestinal effects of DKT have become a vibrant area of clinical and basic research in recent years.

Several animal studies have reported that the ameliorating effects of DKT on laparotomy or chemically induced intestinal dysmotility and postoperative intestinal adhesion were abrogated by atropine, a 5-hydroxytryptamine(4) antagonist (Tokita et al., 2007), and a transient receptor potential-channel antagonist (Tokita et al., 2011), respectively, suggesting that the promotility and antiadhesion effects of DKT likely occur via the activation of 5-hydroxytryptamine(4) receptors and transient receptor potential channel. Further, recent studies have addressed the possibility that DKT increases intestinal blood flow and ameliorates colitis via calcitonin gene-related peptide or adrenomedullin (Murata et al., 2002; Kono et al., 2008, 2010, 2011). The wide range of medicinal actions of DKT has been attributed to its multiple active constituents such as sanshoools, shogaools, and ginsenosides. On the basis of a number of reports indicating the ameliorating effect of DKT in various animal gastrointestinal disease models, several double-blind, placebo-controlled, randomized trials in patients with postoperative paralytic ileus, refractory functional constipation, irritable bowel syndrome, or Crohn’s disease are currently being conducted in Japan (JFMC39-0902, JFMC40-1001, and JFMC42-1002 funded by the Japanese Foundation For Multidisciplinary Treatment of Cancer) and in the United States (NCT00871325, NCT01139216, NCT01388933, and NCT01348152) with the US Food and Drug Administration approval of DKT as an investigational new drug. Among these studies, one recent study reported that DKT has a prokinetic effect in healthy volunteers (Manabe et al., 2010).

Despite widespread use in clinical practice, the pharmacokinetic (PK) knowledge of DKT is limited. Iwabu et al. (2010) reported that 44 compounds derived from DKT were detected in the plasma and urine after oral administration of DKT by using the liquid chromatography-tandem mass spectrometry. Moreover, Munekage et al. (2011) reported...

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ABBREVIATIONS: AUC, area under the curve; BMI, body mass index; BOL, below the quantification limit; CL1/F, oral clearance of the central compartment; CL2/F, intercompartmental clearance; DKT, daikenchuto; GRB1, ginsenoside Rb1; HAS, hydroxy-α-sanshoool; HBS, hydroxy-β-sanshoool; K1, first-order absorption rate constant; PK, pharmacokinetic; RSE%, relative standard error of estimation; 6S, [6]-shogaool; 10S, [10]-shogaool; V1/F, volume of distribution for the central compartment; V2/F, volume of distribution for the peripheral compartment.

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the plasma concentration profiles of six pharmacologically active constituents of DKT—hydroxy-α-sanshool (HAS), hydroxyl-β-sanshool (HBS), 6-shogaol (6S), 10-shogaol (10S), ginsenoside Rb1 (GRB1), and ginsenoside Rg1—in Japanese healthy volunteers.

In recent years, the population PK approach has been used for the development of various pharmaceuticals (Williams and Ette, 2000). This approach can identify the measurable factors that cause changes in the dose-concentration relationship and the extent of these changes (Williams and Ette, 2000). In this study, we first sought to develop the population PK models for the five constituents of DKT using the plasma concentration data obtained from healthy volunteers participating in the Japanese or US study. We then determined whether potential interethnic differences in the PK of DKT existed between the study populations.

Materials and Methods

Clinical Trials and Data Collection. Data were collected from two randomized, open-label, three-arm, three-period crossover studies in Japan and the United States. Participants received single oral doses of 2.5, 5, and 10 g of DKT after fasting for 12 hours at each period. A washout period of greater than 1 month followed period I and period II, preceding the administration of the next dose of the study drug. All foods and drinks (including spices) containing ginseng, Japanese pepper, and ginger were strictly prohibited from 3 days before dispensing the study medication until completion of each treatment phase. Overall study design is summarized in Fig. 1.

In the Japanese study, 19 healthy volunteers enrolled, 18 completed the study, and a total of 560 observations at 0.25, 0.5, 1, 2, 3, 4, 8, 12, 24, and 48 hours after dosing were used for the population PK analysis. Three participants did not meet the eligibility criteria of the study. The effects of these data were evaluated by final population models as sensitivity analysis.

In the US study, 36 healthy volunteers enrolled, 30 completed the study, and a total of 1299 observations at 0.25, 0.5, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96, and 168 hours after dosing were collected. Data from one subject were excluded from the data set for the model building of GRB1 because the concentration-time profile showed a pharmacokinetically unreliable pattern. However, the entire data set was re-evaluated using final population models.

Disposition and demographics of the participants are summarized in Table 1. The content of each DKT constituent in the study medication was comparable between the two studies (Table 2).

Determination of Plasma Concentration of DKT Constituents. The concentrations of five DKT constituents—HAS, HBS, 6S, 10S, and GRB1—were determined by a validated liquid chromatography-tandem mass spectrometry method as reported by Munekage et al. (2011). The limits of the quantification were 0.01 ng/ml for HAS, HBS, and GRB1 and 0.02 ng/ml for 6S and 10S.

Population Pharmacokinetic Model Building. Population PK analysis was performed using the Phoenix NLME (version 1.3; Certara L.P., St. Louis, MO) by the Laplacian method. One- or two-compartment models with or without extravascular input were examined for exploration of the mean structure of the modeling. The basic PK parameters used in this study were oral clearance of the central compartment (CL/F), volume of distribution for the central compartment (V1/F), intercompartmental clearance (CL2/F), volume of distribution for the peripheral compartment (V2/F), and first-order absorption rate constant (K_a). Nonlinear absorption coefficient (b) was introduced for GRB1 population PK modeling as a power of dose (see Supplemental Model Equation 5).
The interoccasion variability and the interindividual variability were modeled by lognormal distribution using eq. 1:

$$P_{ikj} = tvP_{ik} \times \exp\left(\eta_{iocikj} + \eta_{ik}\right)$$

where $P_{ikj}$ is the kth pharmacokinetic parameter for the ith individual during the jth period, $tvP_{ik}$ is the covariate adjusted typical value of the kth parameter, $\eta_{iocikj}$ is a interoccasion variability, and $\eta_{ik}$ is a interindividual variability. $\eta_{ik}$ is Gaussian random deviate for the ith individual in the kth parameter with mean 0 and standard deviation $\sigma_k$. $\eta_{iocikj}$ is an independent Gaussian random deviate with mean 0 and standard deviation $\sigma_{iocikj}$.

Fig. 2. Mean plasma concentration of five daikenchuto constituents in Japanese healthy volunteers. (A) HAS, (B) HBS, (C) 6S, (D) 10S, and (E) GRB1. Each symbol represents mean $\pm$ S.D. of observed concentration. Solid line indicates the model predicted concentration using the median values as covariate.

Fig. 3. Mean plasma concentration of five daikenchuto constituents in US healthy volunteers. (A) HAS, (B) HBS, (C) 6S, (D) 10S, and (E) GRB1. Each symbol represents mean $\pm$ S.D. of observed concentration. Solid line indicates the model predicted concentration using the median values as covariate.
for the $i_{th}$ individual in the $k_{th}$ parameter during the $j_{th}$ period with mean $0$ and standard deviation $\sigma$. 

The residual variability was described by the proportional error model (eq. 2) or combined proportional and additive model (eq. 3). The proportional error model is

$$C_{\text{obs},ijt} = C_{ijt} \times \frac{1 + \text{Eps}_{ijt}}{C_0^{1/71.8}};$$

where $C_{\text{obs},ijt}$ is the plasma concentration observed in the $i_{th}$ individual, at time $t$ after the drug administration during the $j_{th}$ period. $C_{ijt}$ is the predicted plasma concentration, and $\text{Eps}_{ijt}$ is a random variable which is normally distributed with mean 0 and standard deviation $\sigma$.

The combined proportional and additive model is

$$C_{\text{obs},ijt} = C_{ijt} + \text{Eps}_{ijt} \cdot \frac{\text{CMix Ratio}}{C_0^{1/71.8}};$$ 

where the proportional error component is obtained as the product of $\text{Eps}_{ijt}$ and $\text{CMix Ratio}$.

Once the basic model was selected, the influences of covariates were evaluated by a stepwise procedure based on the likelihood ratio test using $P < 0.05$.
as entry criterion. The covariates evaluated were the individual’s age, body weight, body mass index (BMI), gender, and participation in the Japanese or US study (interstudy difference).

The influences of continuous covariates (age, body weight, and BMI) onto the \( k_{ao} \) parameter were described as a power model as shown in eq. 4:

\[
\hat{tvP}_k = tvP_k \cdot \left( \frac{\text{Age}_i}{\text{Median Age}} \right)^{dP_k d\text{Age}} \cdot \left( \frac{\text{WT}_i}{\text{Median WT}} \right)^{dP_k d\text{WT}} \cdot \left( \frac{\text{BMI}_i}{\text{Median BMI}} \right)^{dP_k d\text{BMI}}
\]

(4)

where \( tvP_k \) is a typical value of \( k_{ao} \) parameter; \( \text{WT} \) is the body weight; and \( dP_k d\text{Age} \), \( dP_k d\text{WT} \), and \( dP_k d\text{BMI} \), are the fixed effect parameters for the age, body weight, and BMI.

The influences of categorical covariate (gender and study identifier) were described as eq. 5:

\[
\hat{tvP}_k = tvP_k \cdot \exp(dP_k G_{\text{female}}) \cdot \exp(dP_k S_{\text{US}})
\]

(5)

where \( G_{\text{female}} \) is a dummy variable which took on a value of 1 if the gender of the subject was female and 0 otherwise. Likewise \( S_{\text{US}} \) is a dummy variable which took on a value of 1 if the study was conducted in the United States and 0 otherwise.

The BQL (below the quantification limit) values were treated as the left censored data and used in the model fitting procedure via the maximum likelihood method (Beal, 2001).

**Model Validation.** Bootstrap resampling method (Ette, 1997) and visual predictive check method (Post et al., 2008) were used to evaluate the accuracy and robustness of our models. A total of 1000 resamplings was executed for the bootstrap method, and a total of 1000 replicates of the original data set were simulated for the predictive check method to generate the predicted concentration values and the 95% prediction interval.

**Results**

Demographics and disposition of the study participants are summarized in Table 1. The population PK analysis included a total 1859 samples from 55 participants. There were marked differences in subject demographics.

Figures 2 and 3 demonstrate observed (mean \( +/- \) S.D., presented as dots) and the model predicted (population mean values, presented as solid lines) plasma concentration-time profiles of the five DKT constituents (HAS, HBS, 6S, 10S, and GRB1) after a single oral dose of DKT in healthy Japanese and US adults. Noticeable differences were observed in the mean plasma concentrations of HAS and HBS between the Japanese and US participants.

Final population PK parameters are summarized in Table 3. Population PK model of HAS was best described by a two-compartment model with a bolus input (see Supplemental Model Equation 1). Intercocasion variability and interindividual variability were estimated for \( V1/F \), \( CL1/F \), and \( V2/F \). The interindividual variation of PK

![Fig. 5. Goodness-of-fit plot for HBS. (A) Observations plotted against population predicted concentrations. (B) Observations plotted against individual predicted concentrations.](image-url)

![Fig. 6. Goodness-of-fit plot for 6S. (A) Observations plotted against population predicted concentrations. (B) Observations plotted against individual predicted concentrations.](image-url)
parameters showed a positive correlation. A combined proportional and additive model was selected to describe the residual variability. BMI and age were the covariates affecting V1/F, V2/F, and CL1/F. The relative standard error of estimation (RSE%) for the fixed effect parameters stayed within the range from 2.7 to 32.7%, and the RSE% of random effect parameters ranged from 7.8 to 45.5% (Supplemental Table 1). Goodness-of-fit plot for the final population PK model showed no remarkable biases (Fig. 4). The visual predictive check plots indicated that the predictive concentrations displayed a good fit with the observed concentrations (Supplemental Fig. 1). The resampling successfully converged in the bootstrap evaluation, and the estimated parameters from bootstrap were similar to the parameters obtained from the final model (Supplemental Table 1).

The model for HBS, 6S, and 10S was best described by a one-compartment population PK model with a bolus input (see Supplemental Model Equations 2–4). Interindividual variability was estimated for V1/F and CL1/F. For HBS, BMI and age affected V1/F and CL1/F as covariates. Similarly, body weight was incorporated into V1/F and CL1/F for the 6S model. However, the model for 10S retained no significant covariates. The RSE% for the parameters stayed within the range of 2.7 to 33.2% (Supplemental Tables 2–4). Goodness-of-fit plot for the final population PK model of HBS (Fig. 5) and 6S (Fig. 6) indicated no remarkable biases. The plots of the 10S model implied that the model contained a slight asymmetry at high concentrations (Fig. 7). The visual predictive check plots indicated that there were good agreements between the predicted and observed concentrations (Supplemental Figs. 2, 3, and 4). The resampling successfully converged in the bootstrap evaluation, and the estimated parameters from bootstrap were similar to the parameters obtained from the final model (Supplemental Tables 2–4).

The population the PK model of GRB1 was best described by a one-compartment model with nonlinear extravascular input (see Supplemental Model Equation 5). Interindividual variability was estimated for V1/F, K\textsubscript{a}, and CL/F. The interoccasion variability was calculated for V1/F. No statistically significant covariate was incorporated into the model. For all estimated parameters, RSE% was considered acceptable (within the range of 2.6 to 30.0%), except for the covariance between K\textsubscript{a} and CL1/F, which showed greater RSE% as a result of the mean value being nearly zero (Supplemental Table 5). Residuals of population prediction and the observed value showed log-normal distribution. On the other hand, individual post hoc estimation and observed value showed no remarkable biases (Fig. 8). The visual predictive check plots indicated that the predictive concentrations were well fitted to the observed concentrations (Supplemental Fig. 5). The
proximity of were actually observed during the absorption phase. Without incor-

cretation (i.e., the first sampling point), based on participants who

indicated that the predicted plasma concentrations overestimated the

in the absorption phase, the misspecification of the rate of drug ab-

in the absorption phase, the model is still considered applicable to the

PB characterization of the compound with a short absorption phase.

Although useful information is included in the BQL data, there are

resampling successfully converged in the bootstrap evaluation, and the

estimated parameters from bootstrap were similar to the parameters

from final model (Supplemental Table 5).

Figure 9 indicates that the influence of covariates on the calculated

area under the curve (AUC) of DKT constituents HAS, HBS, and 6S.

BMI showed a pronounced influence on the AUCs of HAS and HBS.

Discussion

We analyzed six pharmacologically active constituents of DKT—

HAS, HBS, 6S, 10S, GRB1, and GRG1 in respective Japanese and US

PK studies. Population PK models were constructed for the five con-

stituents, but not for GRG1, because most of the GRG1 concentrations fell below the quantification limit (BQL).

When the first-order absorption model and the bolus input model were evaluated as population PK models for HAS, HBS, 6S, and 10S, the bolus input model was found to best describe the PK of these constituents. This was because the $t_{\text{max}}$ was observed in many subjects at the first sampling point, which occurred at an early time point of 15 minutes. Wade et al. (1993) reported that when no data are present in the absorption phase, the misspecification of the rate of drug absorption or the model used to describe drug absorption has little consequence on the estimation of the remaining population parameters. On the other hand, the goodness-of-fit plot of the model for 10S indicated that the predicted plasma concentrations oversaturate the observed plasma concentrations at the highest predicted plasma concentration (i.e., the first sampling point), based on participants who were actually observed during the absorption phase. Without incorporating the absorption phase into the model, the plasma level in the proximity of $C_{\text{max}}$ was unpredictable. Although the modeling is limited to an elimination phase, the model is still considered applicable to the PK characterization of the compound with a short absorption phase.

As reported previously in Japanese PK study (Munekage et al., 2011), nonlinearity was observed in AUC of GRB1, but dose-dependence in half-life was not; therefore, a nonlinear absorption model was assumed for the GRB1 analysis. As a result, nonlinear parameter (b) showed a significant value, and the AIC value indicated a better fit compared with the model not assuming the nonlinear parameter. Estimated b value below 1 suggested the convex dose-concentration relationship.

The BQL data included in the dataset were used for the analysis. Although useful information is included in the BQL data, there are concerns regarding the possible bias caused by the mishandling of BQL data. (Hing et al., 2001; Byon et al., 2008). Beal (2001) reported an overview of ways to fit a PK model in the presence of BQL data. The method applied conditional likelihood estimation to the observations above BQL and the likelihood for the data being above the BQL were maximized with respect to the model parameters. Phoenix NLME (Pharsight, St. Louis, MO), which was the analysis software used in this study, implemented this method. We therefore treated BQL data as left censored data and used them in the model fitting procedure via the maximum likelihood method.

Among the covariates evaluated, BMI, age, and body weight affected CL/F and Vd/F for HAS, HBS, and 6S. The three-dimensional plot (Fig. 9) of the covariate relationship with AUC indicated that BMI was the most important covariate to explain the AUC variability of HAS and HBS because the AUCs decreased by 2-fold when the BMI increased by 2-fold from 18 to 30. The package insert of DKT in Japan describes that the dosage may be adjusted according to the patient’s age, body weight, and symptoms, and our findings support this statement. However, it is necessary to judge in consideration of clinical meaning about the necessity for dosage adjustment by more detailed examination, including the clinical study on efficacy and safety.

Remarkable differences were observed in mean plasma concentra-

tions of HAS and HBS between the Japanese and US participants (Figs. 2 and 3). However, interstudy difference has not been selected for the final models of all constituents of DKT. On the other hand, the simulated plasma concentrations at the median value of covariates in each study could reproduce the study difference observed. These results suggest that the difference in the plasma levels between the study populations could be explained by the difference in terms of demographic factors such as BMI and age rather than by interethnic differences between the Japanese and the US habitants.

The likelihood ratio test is frequently used as the criteria for the selection of covariates. The possibility of type I error inflation in the likelihood ratio test has been cautioned (Wählby et al., 2001). Therefore, a very low $P$ value such as $P < 0.001$ is often used as the significance level. Nevertheless, as the first exploratory analysis of DKT via population modeling, we set a criterion of $P = 0.01$ for the logistic analysis.

Data were only used in the development of the model. For the validation of the model, a separate dataset was used in addition to the BQL data, including the data in the clinical study that aimed at efficacy and safety. The method is mainly used for the modeling of oral administration and interstudy difference was not selected for final model. Therefore, we did not use the validation dataset. Instead, we treated BQL data as left censored data and used them in the model fitting procedure via the maximum likelihood method.
Pharmacokinetic information is very useful to characterize a medication and is indispensable to determine the proper use of the medication. However, the clinical effects of herbal medicines are complex because of the presence of numerous constituents. We therefore constructed PK models for five constituents of a single formulation that simultaneously contains constituents with very different PK properties, as seen from constituents with a short half-life, such as shogaols and sanshools, compared with those with a long half-life, such as ginsenosides. To extrapolate our findings effectively to a wider population, further investigation of the relationship between PK and efficacy is warranted.

The results from this study are useful and are a preliminary step toward a more comprehensive pharmacokinetic/pharmacodynamic study in patients.

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Authorship Contributions

Participated in research design: Munekage, Ichikawa, Kitagawa, Kono, Hanazaki.

Conducted experiments: Munekage, Ichikawa, Kitagawa, Hanazaki.

Performed data analysis: Ishihara, Uehara, Watanabe.

Contributed to the writing of the manuscript: Munekage, Ichikawa, Kitagawa, Ishihara, Uehara, Watanabe, Kono, Hanazaki.

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Supplemental material

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List of notation

$C_{i_{th}}$: Estimated plasma concentration in the $i_{th}$ individual, at time $t$ after the drug administration during the $j_{th}$ period.

$C_{obs,i_{th}}$: The plasma concentration observed in the $i_{th}$ individual, at time $t$ after the drug administration during the $j_{th}$ period.

$D_{i_{th}}$: Dose of the $i_{th}$ individual during the $j_{th}$ period.

$CL_{1_{th}}$: Clearance for the central compartment of the $i_{th}$ individual during the $j_{th}$ period.

$CL_{2_{th}}$: Inter-compartmental clearance of the $i_{th}$ individual during the $j_{th}$ period.

$V_{1_{th}}$: Distribution volume for the central compartment of subject $i$ during the $j_{th}$ period.

$V_{2_{th}}$: Distribution volume for the peripheral compartment of subject $i$ during the $j_{th}$ period.

$t_{i_{th}}$: Sampling time in the $i_{th}$ individual, after the drug administration during the $j_{th}$ period.

$K_{a_{th}}$: Absorption rate constant of the $i_{th}$ individual.

$b_{i_{th}}$: Non-linear absorption coefficient of the $i_{th}$ individual.

$BMI_{i_{th}}$, $AGE_{i_{th}}$, $WT_{i_{th}}$: BMI, Age and body weight in the $i_{th}$ individual.

$median(BMI)$, $median(AGE)$, $median(WT)$: Median of BMI, age and body weight of all participants.

$t_{vV1}$, $t_{vV2}$, $t_{vCL1}$, $t_{vCL2}$, $t_{vKa}$ and $t_{vbb}$: Typical value for the each pharmacokinetic parameter.

$nIOCV_{1_{th}}$, $nIOCV_{2_{th}}$, $nIOCCL_{1_{th}}$: Gaussian random deviates for the $i_{th}$ individual during the $j_{th}$ period regarding the pharmacokinetic parameters.

$nV_{1_{th}}$, $nV_{2_{th}}$, $nCL_{1_{th}}$, $nCL_{2_{th}}$, $nK_{a_{th}}$ and $nb_{th}$: Gaussian random deviates for the $i_{th}$ individual regarding the pharmacokinetic parameters.

$\omega_{IOCV}^2_{CL1}$, $\omega_{IOCV}^2_{V1}$ and $\omega_{IOCV}^2_{V2}$: Interoccasion variances of natural-log-transformed PK parameters.

$\omega_{CL1}^2$, $\omega_{V1}^2$, $\omega_{V2}^2$ and $\omega_{ka}^2$: Interindividual variances of natural-log-transformed PK parameters.
\( \omega_{CL1,V1}, \ \omega_{CL1,V2}, \ \omega_{V1,V2}, \ \omega_{Ka,V1}, \ \text{and} \ \omega_{Ka,CL1} \);  
Interindividual covariances of natural-log-transformed PK parameters

\( \sigma^2 \);  
Parameter for intra-individual variability
S-1  PPK Model for HAS

Final Equation

\[ C_{ij,t} = \frac{D_{ij}}{V_{1ij}} (A_{ij} e^{-\alpha_{ij} \cdot t_{ij}} - B_{ij} e^{-\beta_{ij} \cdot t_{ij}}) \]

\[ A_{ij} = \frac{(\alpha_{ij} - K_{21ij})}{(\alpha_{ij} - \beta_{ij})} \]

\[ B_{ij} = \left( \frac{\beta_{ij} - K_{21ij})}{(\beta_{ij} - \alpha_{ij})} \right. \]

\[ \alpha_{ij} = \frac{1}{2} \left( (K_{12ij} + K_{21ij} + K_{eij}) + \sqrt{(K_{12ij} + K_{21ij} + K_{eij})^2 - 4 \cdot K_{21ij} \cdot K_{eij}} \right) \]

\[ \beta_{ij} = \frac{1}{2} \left( (K_{12ij} + K_{21ij} + K_{eij}) - \sqrt{(K_{12ij} + K_{21ij} + K_{eij})^2 - 4 \cdot K_{21ij} \cdot K_{eij}} \right) \]

\[ K_{12ij} = \frac{CL_{2ij}}{V_{1ij}} \]

\[ K_{21ij} = \frac{CL_{2ij}}{V_{2ij}} \]

\[ K_{eij} = \frac{CL_{1ij}}{V_{1ij}} \]

\[ CL_{1ij} = tv_{CL1} \cdot \left\{ \frac{(BM_{ij})}{(med\text{median}(BM))} \left( dCL_{1dBM} \right) \right\} \cdot \left\{ \frac{(AGE_{ij})}{(med\text{median}(AGE))} \left( dCL_{1dAGE} \right) \right\} \cdot e^{hCL_{1i}} \cdot e^{nOCCL_{1ij}} \]

\[ V_{1ij} = tv_{V1} \cdot \left\{ \frac{(BM_{ij})}{(med\text{median}(BM))} \left( dV_{1dBM} \right) \right\} \cdot \left\{ \frac{(AGE_{ij})}{(med\text{median}(AGE))} \left( dV_{1dAGE} \right) \right\} \cdot e^{nV_{1i}} \cdot e^{nOCV_{1ij}} \]

\[ CL_{2ij} = tv_{CL2} \]

\[ V_{2ij} = tv_{V2} \cdot \left\{ \frac{(BM_{ij})}{(med\text{median}(BM))} \left( dV_{2dBM} \right) \right\} \cdot \left\{ \frac{(AGE_{ij})}{(med\text{median}(AGE))} \left( dV_{2dAGE} \right) \right\} \cdot e^{nV_{2i}} \cdot e^{nOCV_{2ij}} \]

\[ \begin{bmatrix} n_{\text{LOCCL}_{1ij}} \\ n_{\text{OCV}_{1ij}} \\ n_{\text{OCV}_{2ij}} \end{bmatrix} \sim N \left( \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \omega_{\text{LOCCL}_{1i}}^2 & 0 & 0 \\ 0 & \omega_{\text{OCV}_{1i}}^2 & 0 \\ 0 & 0 & \omega_{\text{OCV}_{2i}}^2 \end{bmatrix} \right) \]

\[ \begin{bmatrix} n_{\text{CL1}_{i}} \\ n_{\text{V1}_{i}} \\ n_{\text{V2}_{i}} \end{bmatrix} \sim N \left( \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \omega_{\text{CL1}_{i}}^2 & \omega_{\text{CL1}_{i} \text{V1}_{i}} & \omega_{\text{CL1}_{i} \text{V2}_{i}} \\ \omega_{\text{CL1}_{i} \text{V1}_{i}} & \omega_{\text{V1}_{i}}^2 & \omega_{\text{V1}_{i} \text{V2}_{i}} \\ \omega_{\text{CL1}_{i} \text{V2}_{i}} & \omega_{\text{V1}_{i} \text{V2}_{i}} & \omega_{\text{V2}_{i}}^2 \end{bmatrix} \right) \]

\[ C_{\text{obs,ijt}} = C_{ij,t} + E_{\text{psijt}} \left( 1 + C_{ij,t} \cdot CMixRatio \right) \]

\[ E_{\text{psijt}} \sim N(0, \sigma^2) \]
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE%</th>
<th>Estimate</th>
<th>RSE%</th>
<th>95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{V1}$  (L/hr)</td>
<td>0.0343</td>
<td>6.8</td>
<td>0.0345</td>
<td>13.0</td>
<td>0.0267 - 0.0442</td>
</tr>
<tr>
<td>$t_{V2}$  (L/hr)</td>
<td>0.281</td>
<td>6.4</td>
<td>0.286</td>
<td>7.8</td>
<td>0.241 - 0.330</td>
</tr>
<tr>
<td>$dCldAge$</td>
<td>0.322</td>
<td>27.5</td>
<td>0.330</td>
<td>38.2</td>
<td>0.0961 - 0.576</td>
</tr>
<tr>
<td>$dVdBMI$</td>
<td>0.828</td>
<td>32.7</td>
<td>0.823</td>
<td>63.2</td>
<td>-0.297 - 1.75</td>
</tr>
<tr>
<td>$ω_{loc}^{2} CL1/F$</td>
<td>0.0254</td>
<td>16.7</td>
<td>0.0235</td>
<td>24.2</td>
<td>0.00950 - 0.0337</td>
</tr>
<tr>
<td>$ω_{loc}^{2} V1/F$</td>
<td>0.0194</td>
<td>20.0</td>
<td>0.0171</td>
<td>34.7</td>
<td>0.00283 - 0.0279</td>
</tr>
<tr>
<td>$ω_{loc}^{2} V2/F$</td>
<td>0.130</td>
<td>26.4</td>
<td>0.117</td>
<td>50.3</td>
<td>0.000808 - 0.238</td>
</tr>
<tr>
<td>$σ_{Additive}$ (ng/mL)</td>
<td>0.00481</td>
<td>7.8</td>
<td>0.00475</td>
<td>7.6</td>
<td>0.00402 - 0.00546</td>
</tr>
<tr>
<td>CmixRatio</td>
<td>44.8</td>
<td>8.5</td>
<td>46.0</td>
<td>10.3</td>
<td>38.3 - 56.5</td>
</tr>
<tr>
<td>$σ_{Proportional}$ (%)</td>
<td>21.6</td>
<td>N.C</td>
<td>21.8</td>
<td>5.1</td>
<td>19.7 - 24.0</td>
</tr>
</tbody>
</table>

N.C.; Not Calculated.
Fig. 1 Predictive Check Plot for HAS

● ; Observation

--- ; Observed Quantiles (5%, 50%, 95%)

----- ; Predicted Quantiles (5%, 50%, 95%)
S-2  PPK Model for HBS

Final Equation

\[ C_{i,j,t} = \frac{D_{i,j}}{V_{1,ij}} e^{-K_{e_{ij}} t_{ij}} \]

\[ K_{e_{ij}} = \frac{CL_{1,ij}}{V_{1,ij}} \]

\[ CL_{1,ij} = tvCL1 \cdot \left\{ \frac{(BMI_i)}{(median(BMI))} \right\}^{(dCL1dBMI)} \cdot \left\{ \frac{(AGE_i)}{(median(AGE))} \right\}^{(dCL1dAGE)} \cdot e^{nCL_{1,i}} \cdot e^{nIOCCL_{1,ij}} \]

\[ V_{1,ij} = tvV1 \cdot \left\{ \frac{(BMI_i)}{(median(BMI))} \right\}^{(dV1dBMI)} \cdot \left\{ \frac{(AGE_i)}{(median(AGE))} \right\}^{(dV1dAGE)} \cdot e^{nV_{1,i}} \cdot e^{nIOCV_{1,ij}} \]

\[
\begin{bmatrix}
[nIOCCL_{1,ij}] \\
[nIOCV_{1,ij}]
\end{bmatrix}
\sim N\left(\begin{bmatrix}0 \\0\end{bmatrix}, \begin{bmatrix}
\omega_{IOCCCL_{11}}^{2} & 0 \\
0 & \omega_{IOCCV_{11}}^{2}
\end{bmatrix}\right)
\]

\[
\begin{bmatrix}
[nCL_{1,i}] \\
[nV_{1}]
\end{bmatrix}
\sim N\left(\begin{bmatrix}0 \\0\end{bmatrix}, \begin{bmatrix}
\omega_{CL1}^{2} & \omega_{CL1,V_{1}} \\
\omega_{CL1,V_{1}} & \omega_{V_{1}}^{2}
\end{bmatrix}\right)
\]

\[ C_{obs,i,j,t} = C_{i,j,t} + Eps_{ijt} \left(1 + C_{i,j,t} \cdot CMixRatio\right) \]

\[ Eps_{ijt} \sim N(0, \sigma^2) \]
Table 2 Final population parameter of HBS and the results of bootstrap validation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimates from Final Model</th>
<th>Estimates from Bootstrap simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>RSE%</td>
</tr>
<tr>
<td>tvCL1 (L/hr)</td>
<td>6.95</td>
<td>4.3</td>
</tr>
<tr>
<td>tvV1 (L)</td>
<td>14.4</td>
<td>3.6</td>
</tr>
<tr>
<td>dCldAge</td>
<td>0.389</td>
<td>30.1</td>
</tr>
<tr>
<td>dVdAge</td>
<td>0.560</td>
<td>19.4</td>
</tr>
<tr>
<td>dCldBMI</td>
<td>1.37</td>
<td>22.1</td>
</tr>
<tr>
<td>dVdBMI</td>
<td>2.19</td>
<td>12.3</td>
</tr>
<tr>
<td>$\omega_{ico}^2_{CL1}$</td>
<td>0.0818</td>
<td>14.0</td>
</tr>
<tr>
<td>$\omega_{ioC}^2_{V1}$</td>
<td>0.0579</td>
<td>15.2</td>
</tr>
<tr>
<td>$\omega^2_{CL1/F}$</td>
<td>0.0641</td>
<td>27.1</td>
</tr>
<tr>
<td>$\omega^2_{V1/F}$</td>
<td>0.0427</td>
<td>25.5</td>
</tr>
<tr>
<td>$\omega_{V1/F,CL1/F}$</td>
<td>0.0520</td>
<td>21.8</td>
</tr>
<tr>
<td>$\sigma_{Additive}$ (ng/mL)</td>
<td>0.0125</td>
<td>5.0</td>
</tr>
<tr>
<td>CmixRatio</td>
<td>16.9</td>
<td>5.8</td>
</tr>
<tr>
<td>$\sigma_{Proportional}$ (%)</td>
<td>21.1</td>
<td>N.C.</td>
</tr>
</tbody>
</table>

N.C.; Not Calculated.
Fig. 2 Predictive Check Plot for HBS

● ; Observation

- ; Observed Quantiles (5%, 50%, 95%)

- - - ; Predicted Quantiles (5%, 50%, 95%)
S-3  PPK Model for 6S

Final Equation

\[ C_{ij,t} = \frac{D_{ij}}{V_{ij}} e^{-K_{ei}t_{ij}} \]

\[ K_{ei} = \frac{CL_{1ij}}{V_{ij}} \]

\[ CL_{1ij} = tvCL1 \cdot \left\{ \frac{(WT_{i})}{\text{median}(WT)} \right\}^{(dCL1dWT)} \cdot e^{nCL1} \]

\[ V_{1ij} = tvV1 \cdot \left\{ \frac{(WT_{i})}{\text{median}(WT)} \right\}^{(dV1dWT)} \cdot e^{nV1} \]

\[
\begin{bmatrix}
    nCL1_i \\
    nV1_i
\end{bmatrix}
\sim N\left( \begin{bmatrix}
    0 \\
    0
\end{bmatrix},
\begin{bmatrix}
    \sigma^2_{CL1} & \sigma_{CL1,V1} \\
    \sigma_{CL1,V1} & \sigma^2_V
\end{bmatrix}\right)
\]

\[ C_{obs,ij,t} = C_{ij,t} + \varepsilon_{ij,t} \left( 1 + C_{ij,t} \cdot \text{CMixRatio} \right) \]

\[ \varepsilon_{ij,t} \sim N(0, \sigma^2) \]
Table 3 Final population parameter of 6S and the results of bootstrap validation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimates from Final Mode</th>
<th>Estimates from Bootstrap simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>RSE%</td>
</tr>
<tr>
<td>tvCL1 (L/hr)</td>
<td>8451</td>
<td>4.3</td>
</tr>
<tr>
<td>tvV1 (L)</td>
<td>4259</td>
<td>5.9</td>
</tr>
<tr>
<td>dCl/dWt</td>
<td>0.841</td>
<td>23.4</td>
</tr>
<tr>
<td>dV/dWt</td>
<td>0.933</td>
<td>28.3</td>
</tr>
<tr>
<td>$\omega_{CL1/F}$</td>
<td>0.0715</td>
<td>25.0</td>
</tr>
<tr>
<td>$\omega_{V1/F}$</td>
<td>0.0942</td>
<td>33.2</td>
</tr>
<tr>
<td>$\omega_{V1/F,CL1/F}$</td>
<td>0.0648</td>
<td>31.7</td>
</tr>
<tr>
<td>$\sigma_{Additive}$ (ng/mL)</td>
<td>0.00558</td>
<td>10.4</td>
</tr>
<tr>
<td>CMixRatio</td>
<td>63.8</td>
<td>13.3</td>
</tr>
<tr>
<td>$\sigma_{Proportional}$ (%)</td>
<td>35.7</td>
<td>N.C.</td>
</tr>
</tbody>
</table>

N.C.; Not Calculated.
Fig. 3 Predictive Check Plot for 6S

- ●: Observation
- -: Observed Quantiles (5%, 50%, 95%)
- ---: Predicted Quantiles (5%, 50%, 95%)
S-4  PPK Model for 10S

Final Equation

\[ C_{i,j,t} = \frac{D_{ij}}{V_{1ij}} e^{-K_{e_{ij}} t_{ij}} \]

\[ K_{e_{ij}} = \frac{CL_{1ij}}{V_{1ij}} \]

\[ CL_{1ij} = tvCL1 \cdot e^{nCL1_{i}} \]

\[ V_{1ij} = tvV1 \cdot e^{nV1_{i}} \]

\[
\begin{bmatrix}
  nCL1_{i} \\
  nV1_{i}
\end{bmatrix}
\sim N\left( \begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix}
  \omega^{2}_{CL1} & \omega_{CL1,V1} \\
  \omega_{CL1,V1} & \omega^{2}_{V1}
\end{bmatrix} \right) \]

\[ C_{obs,ijt} = C_{i,j,t} + \varepsilon_{ijt} \left( 1 + C_{i,j,t} \cdot CMixRatio \right) \]

\[ \varepsilon_{ijt} \sim N(0, \sigma^{2}) \]
Table 4 Final population parameter of 10S and the results of bootstrap validation

<table>
<thead>
<tr>
<th></th>
<th>Estimates from Final Model</th>
<th>Estimates from Bootstrap simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>RSE%</td>
</tr>
<tr>
<td>tvCL1 (L/hr)</td>
<td>279</td>
<td>6.7</td>
</tr>
<tr>
<td>tvV1 (L)</td>
<td>309</td>
<td>6.8</td>
</tr>
<tr>
<td>$\omega^2_{CL1/F}$</td>
<td>0.219</td>
<td>20.8</td>
</tr>
<tr>
<td>$\omega^2_{V1/F}$</td>
<td>0.195</td>
<td>24.5</td>
</tr>
<tr>
<td>$\omega_{V1/F,CL1/F}$</td>
<td>0.151</td>
<td>26.9</td>
</tr>
<tr>
<td>$\sigma_{Additive}$ (ng/mL)</td>
<td>0.0115</td>
<td>3.9</td>
</tr>
<tr>
<td>CmixRatio</td>
<td>47.0</td>
<td>2.7</td>
</tr>
<tr>
<td>$\sigma_{Proportional}$ (%)</td>
<td>54.1</td>
<td>N.C.</td>
</tr>
</tbody>
</table>

N.C.; Not Calculated.
Fig. 4 Predictive Check Plot for 10S

- ●; Observation
- ---; Observed Quantiles (5%, 50%, 95%)
- .......; Predicted Quantiles (5%, 50%, 95%)
S-5  PPK Model for GRB1

Final Equation

\[ C_{i,j,t} = \frac{K_{a,i} \cdot D_{i,j}^{b_i}}{V_{1,ij} \cdot (K_{a,i} - K_{e,i})} \left( e^{-K_{e,i}t_{i,j}} - e^{-K_{a,i}t_{i,j}} \right) \]

\[ K_{e,i} = \frac{CL_{1,ij}}{V_{1,ij}} \]

\[ CL_{1,ij} = tvCL1 \cdot e^{nCL_{1,i}} \]

\[ V_{1,ij} = tvV1 \cdot \left\{ \frac{(AGE_i)}{\text{median}(AGE)} \right\}^{(dV1dAGE)} \cdot e^{nV1} \cdot e^{nOCV_{1,j}} \]

\[ b_i = tvb1 \]

\[ K_{a,i} = tvK_{a1} \cdot \left\{ \frac{(WT_i)}{\text{median}(WT)} \right\}^{(dKadWT)} \cdot e^{nK_{a,i}} \]

\[ nOCV_{1,j} \sim N(0, \omega_{IOCV_{1},j}) \]

\[
\begin{bmatrix}
  nK_{a,i} \\
  nV_{1,i} \\
  nCL_{1,i}
\end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\
  \omega_{K_{a},V_{1}} \\
  \omega_{K_{a},CL_{1}} \end{bmatrix}, \begin{bmatrix} \omega_{K_{a}}^2 & \omega_{K_{a},V_{1}} & \omega_{K_{a},CL_{1}} \\
  \omega_{K_{a},V_{1}} & \omega_{V_{1}}^2 & \omega_{V_{1},CL_{1}} \\
  \omega_{K_{a},CL_{1}} & \omega_{V_{1},CL_{1}} & \omega_{CL_{1}}^2 \end{bmatrix}\right) \]

\[ C_{\text{obs},i,j,t} = C_{i,j,t} \cdot (1 + Eps_{i,j,t}) \]

\[ Eps_{i,j,t} \sim N(0, \sigma^2) \]
Table 5: Final population parameter of GRB1 and the results of bootstrap validation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimates from Final Model</th>
<th>Estimates from Bootstrap simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>RSE%</td>
</tr>
<tr>
<td>tvCL1 (L/hr)</td>
<td>66.2</td>
<td>15.0</td>
</tr>
<tr>
<td>tvV1 (L)</td>
<td>4384</td>
<td>16.2</td>
</tr>
<tr>
<td>Ka (1/hr)</td>
<td>0.719</td>
<td>10.8</td>
</tr>
<tr>
<td>b</td>
<td>0.862</td>
<td>2.6</td>
</tr>
<tr>
<td>ωioc^2\text{V1/F}</td>
<td>0.132</td>
<td>15.2</td>
</tr>
<tr>
<td>ω^2Ka</td>
<td>0.505</td>
<td>20.0</td>
</tr>
<tr>
<td>ω^2CL1/F</td>
<td>0.206</td>
<td>20.1</td>
</tr>
<tr>
<td>ω^2V1/F</td>
<td>0.275</td>
<td>23.1</td>
</tr>
<tr>
<td>ωKa,V1/F</td>
<td>0.295</td>
<td>23.3</td>
</tr>
<tr>
<td>ωKa,CL1/F</td>
<td>0.00523</td>
<td>857.4</td>
</tr>
<tr>
<td>ωV1/F,CL1/F</td>
<td>0.131</td>
<td>30.0</td>
</tr>
<tr>
<td>σ\text{Proportional} (CV%)</td>
<td>32.0</td>
<td>2.2</td>
</tr>
</tbody>
</table>
Fig. 5 Predictive Check Plot for GRB1

- ● : Observation
- --- : Observed Quantiles (5%, 50%, 95%)
- ------- : Predicted Quantiles (5%, 50%, 95%)