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Regular article

Prediction of inter-individual variability on the pharmacokinetics of CYP1A2 substrates in non-smoking healthy volunteers

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ABSTRACT

The activity of CYP1A2, a major drug-metabolizing enzyme, is known to be affected by various environmental factors. Our study aimed to predict inter-individual variability of AUC/Dose of CYP1A2 substrates in non-smoking healthy volunteers using the Monte Carlo simulation. Inter-individual variability in hepatic intrinsic clearance of CYP1A2 substrates ($CL_{int,h,1A2}$) was estimated using dispersion model based on the inter-individual variability (N = 96) of the AUC of caffeine, a major CYP1A2 substrate. The estimated coefficient of variation (CV) of $CL_{int,h,1A2}$ was 55%, similar to previously reported $CL_{int,h,2D6}$ (60%) but larger than $CL_{int,h,3A4}$ (33%). Then, this estimated CV was validated by predicting the CVs of AUC/Dose of tizanidine and phenacetin, which are mainly metabolized by CYP1A2 and have negligible renal clearance. As a result, reported CVs were successfully predicted within 2.5–97.5 percentile range of predicted values. Moreover, CVs for AUC/Dose of the CYP1A2 substrates theophylline and lidocaine, which are affected by other CYPs and renal clearance, were also successfully predicted. The inter-individual variability of AUC/Dose of CYP1A2 substrates was successfully predicted using 55% CV for $CL_{int,h,1A2}$, and the results, along with those reported by our group for other CYPs, support the prediction of inter-individual variability of pharmacokinetics in the clinical setting.

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1. Introduction

In drug development, it is important to predict the clearance of a clinical candidate drug in human, and various methodologies for the prediction of clearance have been reported [1–5]. Generally, a mean value for clearance is predicted, and subsequently the accuracy for the prediction is evaluated by predicting the results of one clinical trial. However, in actuality, clearance in human has inter-individual variability, which, if large, may result in unexpected side effects and failure of the treatment. If the inter-individual variability in the pharmacokinetics of a clinical candidate drug in clinical trials can be predicted beforehand, it will greatly contribute to selecting the optimal clinical candidate.

Previously, our group proposed a methodology using the Monte Carlo simulation to predict the inter-individual variability of the AUC for CYP3A4, CYP2C19, and CYP2D6 substrates and the urine metabolic ratio (MR) for CYP2D6 substrates from the mean value and the variability of physiological parameters and pharmacokinetic parameters that had been collected from literature. Kato et al. successfully predicted the inter-individual variability of the AUC for CYP3A4 substrates by setting the CV of $CL_{int,h}$ of CYP3A4 at 33%, after comparing the reported CV of CYP3A4 content in human liver microsome and the reported CV of AUC of CYP3A4 substrates [6]. Ito et al. estimated the CV of $CL_{int,h}$ of CYP2D6 substrates for an extensive metabolizer (EM) and an intermediate metabolizer (IM) as 60% from the variability in urine MR of CYP2D6 substrates [7], and successfully predicted the inter-individual variability in urine MR of CYP2D6 substrates from the estimated 60% CV. Also, Chiba et al. estimated the CV of $CL_{int,h}$ of CYP2D6 substrates for each CYP2D6 polymorphism from the variability in urine MR of CYP2D6 substrates and successfully predicted the inter-individual variability of AUC of CYP2D6 substrates for each race [8].

In this study, we focused on the inter-individual variability of the AUC of CYP1A2 substrates. CYP1A2 is a major hepatic CYP [9] and its activity has been known to be affected by various polymorphisms and environmental factors [10–13]. Therefore, large inter-individual

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variability in the $CL_{int,h}$ of CYP1A2 substrates can be expected. In particular, smoking is a typical environmental factor that induces the activity of CYP1A2 [14–17] and was reported to reduce the AUC of well-known CYP1A2 substrates caffeine and tizanidine by approximately 50% [18,19]. However, in most cases, detailed information on smoking behavior (frequency of smoking, duration of smoking, etc.) was not described in the literature, so the effect of smoking could not be incorporated into the inter-individual variability of $CL_{int,h}$ of CYP1A2 substrates. Therefore, in this study the inter-individual variability in the AUC of CYP1A2 substrates was predicted in non-smokers.

In this study, caffeine, tizanidine, and phenacetin were selected as typical CYP1A2 substrates. First, the CV of $CL_{int,h}$ of CYP1A2 substrates was estimated from the AUC/Dose (AUC/D) data for caffeine, to which the first-pass effect contributes slightly after oral administration. Then, using the estimated CV, the inter-individual variability in the AUC/D of tizanidine and phenacetin was predicted. Moreover, the accuracy of the predicted value was confirmed by predicting the inter-individual variability of the AUC/D of the CYP1A2 substrates theophylline, which is affected by renal clearance, and lidocaine, which is affected by other CYPs.

2. Materials and method

2.1. Selection of CYP1A2 substrates

Caffeine, tizanidine, and phenacetin were selected as major CYP1A2 substrates that are mainly metabolized in liver and have more than 0.9 of contribution of CYP1A2 in liver metabolism ($f_{m\ CYP1A2}$). Theophylline and lidocaine were selected as CYP1A2 substrates that have contribution of renal clearance and other CYPs.

2.2. Data collection

The mean value and variability (standard deviation (SD), standard error (SE), coefficient of variation (CV), confidence interval (CI)) of physiological parameters (liver volume, hepatic blood flow rate, body weight, and albumin concentration) were collected from literature. The mean value and variability of pharmacokinetic parameters (AUC/D, plasma unbound fraction (f_p), renal clearance (CL_r), ratio of blood-to-plasma concentration (R_B), and excretion fraction of drug in urine (A_e)) of CYP1A2 substrates were also collected from literature (Tables 1 and 2). Since the R_b of tizanidine has not been reported in literature, it was estimated from f_p using Uchimura et al.'s method [20]. Only AUC/D data after a single oral administration in healthy non-smokers were used. Since the ratio of renal clearance in systemic clearance for tizanidine, phenacetin, and lidocaine was below 1%, it was assumed that renal clearance was negligible. PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) was used to search the literature.

When multiple AUC/D data were available for a substrate, the overall mean value and SD were estimated from each mean value and SD using the following equations.

Weighted mean (WM) was calculated from

$$WM = \frac{\sum_{i=1}^{n}(N_i \cdot m_i)}{\sum_{i=1}^{n} N_i}$$

Overall SD was calculated from

$$SD = \sqrt{\frac{\sum_{i=1}^{n}\left[\left\{(SD_i)^2 \times N_i \cdot (N_i - 1) + (N_i \cdot m_i)^2\right\} \Big/ N_i\right] - WM^2 \bullet \sum_{i=1}^{n} N_i}{\sum_{i=1}^{n} N_i - 1}}$$

where N_i is the number of subjects, m_i is the mean value in the ith study, and SD_i is the SD value in the ith study.

2.3. Estimation of $f_{m\ CYP1A2}$ for CYP1A2 substrates

The $f_{m\ CYP1A2}$ was estimated using furafylline [21,22], a known selective CYP1A2 inhibitor, in the following way. In the case of substrate which has a small contribution of renal clearance, more than 0.9 of $f_{m\ CYP1A2}$, and information of AUC changed by furafylline in human, $f_{m\ CYP1A2}$ was estimated from following equation.

$$f_{m\ CYP1A2} = 1 - \frac{AUC}{AUC_f}$$

where AUC_f is the AUC of the CYP1A2 substrate dosed concomitantly with furafylline. When an increase above 900% in the AUC of the CYP1A2 substrate was observed for concomitant dosing, it was assumed that the activity of CYP1A2 was completely inhibited by furafylline. In fact, because reports gave an increase of over 900% in the AUCs of caffeine and phenacetin dosed concomitantly with furafylline [23,24], the values for $f_{m\ CYP1A2}$ of caffeine and phenacetin were estimated as 1. For substrates to which the contribution of renal clearance is large or for which concomitant dosing with furafylline increased the AUC either by less than 900% or there was no report of a change in AUC, $f_{m\ CYP1A2}$ was estimated from *in vitro* CYP1A2 inhibition data or recombinant CYP metabolism data. The $f_{m\ CYP1A2}$ values of tizanidine, theophylline, and lidocaine were estimated in this way. Since approximately 90% of tizanidine metabolism was inhibited by furafylline in an *in vitro* study [25], the $f_{m\ CYP1A2}$ of tizanidine was estimated as 0.9. When $f_{m\ CYP1A2}$ was 0.9

Table 1
Pharmacokinetic parameters of CYP1A2 substrates used in this study.

Substrate	f_p	R_B	$f_{m,\ 1A2}$	CL_r mean (ml/min)	CL_r CV (%)	A_e (%)	Reference
Caffeine	0.8	1	1	1.85	36.3	2	[20,23,60]
Tizanidine	0.7	0.85[a]	1	–[b]	–[b]	<1	[25,61,62]
Phenacetin	0.47	1.01	1	–[b]	–[b]	<1	[24,61,63,64]
Theophylline	0.44	0.82	0.65	8.8	26.1	17	[20,26,65]
Lidocaine	0.296	0.84	0.68	–[b]	–[b]	<1	[27,63,66]

[a] Calculated using the reported method.
[b] Urinary excretion < 1%.

Table 2
Weighted AUC/D and CV values obtained from literature of CYP1A2 substrates.

Substrate	Number of groups	Number of subjects	AUC/D mean (min/l)	AUC/D CV (%)	$CL_{int,h}$ (ml/min/kg)	Reference
Caffeine	9	96	13.7	53.9	1.33	[18,67–72]
Tizanidine	10	167	0.105	86.0	85.0	[19,73–80]
Phenacetin	4	61	0.679	80.2	40.6	[81–83]
Theophylline	13	158	18.7	32.8	1.42	[65,84–94]
Lidocaine	6	45	0.362	52.1	88.3	[95–100]

and over, $f_{m\ CYP1A2}$ was assumed as 1 in this study. The $f_{m\ CYP1A2}$ and $f_{m\ other\ CYPs}$ for theophylline [26] and lidocaine [27] were estimated using normalized intrinsic clearance (CL_{int}) from the reported expression data for each CYP in human liver microsomes (HLM) in an *in vitro* recombinant CYPs metabolism study [28].

2.4. Estimation of variability of $CL_{int,h,1A2}$ from caffeine data

The CV of $CL_{int,h,1A2}$ was estimated by a Monte Carlo simulation using data on the WM of AUC/D and CV of caffeine, which were collected from literature (Fig. 1). The dispersion model was used for the Monte Carlo simulation. One thousand sets of the mean value and CV of the AUC/D were generated using each pharmacokinetic parameter of caffeine and an arbitrary CV of $CL_{int,h,1A2}$. Since AUC/D of caffeine of 96 subjects was collected from literature, 96 of AUC/D were generated in 1 set of simulation. The optimal CV of $CL_{int,h,1A2}$ was estimated by finding comparable CV in simulation with weighted mean of CV in literatures.

AUC/D after oral administration was calculated from the following equation.

$$AUC/Dose = \frac{F_a \cdot F_g \cdot F_h}{CL_h + CL_r}$$

where F_a and F_g were assumed to be 1 and to have no inter-individual variability.

F_h was calculated using the dispersion model from the following equation.

$$Fh = \frac{4a}{(1+a)^2 \cdot \exp\{(a-1)/2/D_N\} - (1-a)^2 \cdot \exp\{-(a+1)/2/D_N\}}$$

$$a = (1 + 4R_N \cdot D_N)^{1/2}$$

$$R_N = f_B \cdot \frac{CL_{int,h}}{Qh}$$

where f_B is the blood unbound fraction, Q_h is the hepatic blood flow rate, and D_N is the dispersion number. D_N was assumed to be 0.17 [29,30].

CL_h was calculated from the following equation.

$$F_h = 1 - \frac{CL_h}{Q_h}$$

The plasma unbound fraction (f_p) was calculated from the equation $f_p = 1/(1 + nPt/K_d)$, where n is the number of binding sites, Pt is the albumin concentration, and K_d is the dissociation constant, and n and K_d were assumed to have no inter-individual variability. The simulation used reported values for Q_h (1.22 mL/min/mL liver, CV: 12.9%) [31], liver volume (19.5 mL/kg, CV: 11.4%) [31], albumin concentration (42.4 g/L, CV: 10.3%) [32–35], and body weight (Asian: 66.2 kg, CV: 12.4%, Caucasian: 78.8 kg, CV: 11.7%) [36].

The Monte Carlo simulation was performed in a virtual population of 96 subjects and generated 1000 sets of AUC/D and CV, and a log-normal distribution of $CL_{int,h}$ was assumed. The simulation was conducted according to Kato et al.'s method and used the RAND function of Microsoft Excel 2007 to generate uniform random numbers ranging from 0 to 1. Two normal random numbers (Z_i and Z_{i+1}) were generated from the following equations.

$$Z_i = (-2*\ln u_i)^{0.5} * \cos(2*\pi*u_{i+1})$$

$$Z_{i+1} = (-2*\ln u_i)^{0.5} * \sin(2*\pi*u_{i+1})$$

No correlation among parameters was assumed.

Above simulation was programmed using Visual Basic for Applications (VBA) of Microsoft Excel 2007, and the Monte Carlo simulation was conducted by generating an arbitrary distribution of each parameter from generated random numbers. All simulation was conducted under assumption of central limit theorem.

2.5. Simulation of AUC/D of CYP1A2 substrates

A Monte Carlo simulation was conducted to generate 1000 sets of the mean value and CV of AUC/D for each CYP1A2 substrate using the dispersion model in a virtual population with the number of subjects which were collected from literature. When the information on R_B was not available in literature, it was estimated from f_p using the reported method [20]. Since Kato et al. reported the CV of $CL_{int,h,3A4}$ to be 33%, this value was used in this study. Since the CV of $CL_{int,h,2E1}$ has not been reported, it was estimated from data on chlorzoxazone, which is a CYP2E1 specific substrate, using the same method as that used to estimate the CV of $CL_{int,h,1A2}$. Compared with other CYPs for which our group has predicted the inter-individual variability, CYP2E1 is a minor isotype, which means there are only a few substrates that are mainly catalyzed by CYP2E1 and data sources for them were limited. Therefore, in this study, the CV of $CL_{int,h,2E1}$ was only estimated from the data of chlorzoxazone. Chlorzoxazone has been used *in vitro* and *in vivo* to prove the presence of CYP2E1 [37,38]. In addition, the urinary excretion of unchanged drug for chlorzoxazone is below 1%, its clearance is low, and its $f_{m\ CYP2E1}$ is 1. Therefore, chlorzoxazone can be considered as the optimal substrate to estimate the CV of $CL_{int,h,2E1}$. The f_p, R_B, and $f_{m\ CYP2E1}$ were set as 0.04, 0.51, and 1 respectively based on information from literature [39,40]. Since its urinary excretion of unchanged drug is below 1%, renal clearance was set as 0 [41].

The log-normal distribution of $CL_{int,h}$ was assumed. The log-transferred mean (μ) and SD (σ) were calculated from the arithmetic mean and CV collected from literatures using the following equations.

$$\mu = \ln(arithmetic\ mean) - \frac{\sigma^2}{2}$$

$$\sigma = \sqrt{\ln\left(\left(\frac{CV}{100}\right)^2 + 1\right)}$$

To confirm the effect of changes in $CL_{int,h}$ on the AUC/D and its CV, the AUC/D and CV were simulated using the pharmacokinetic parameters of caffeine under different $CL_{int,h}$ values (Fig. 4A). It was assumed that 100% of drug was metabolized in the liver, so the simulation was conducted by removing renal clearance from the pharmacokinetic parameters of caffeine. The Monte Carlo simulation generated 1000 sets in a virtual population of 96 subjects using 33% and 55% CV for $CL_{int,h}$.

3. Results

3.1. Estimation of pharmacokinetic parameters for CYP1A2 substrates

The pharmacokinetic parameters of 5 CYP1A2 substrates estimated from literature are listed in Table 1. The $f_{m\ CYP1A2}$ of caffeine, tizanidine, and phenacetin was estimated as 0.96, 0.90, and 0.99 respectively by assuming that the activity of CYP1A2 was completely inhibited by furafylline. If AUC of CYP1A2 substrates increased 10 fold by concomitant dosing with furafylline, $f_{m\ CYP1A2}$ was calculated as 0.90. However, if the concentration of furafylline was insufficient to inhibit the CYP1A2 activity completely, it can be considered that $f_{m\ CYP1A2}$ is above 0.90; therefore, the values for $f_{m\ CYP1A2}$ of caffeine, tizanidine, and phenacetin were set as 1.

The $f_{m\ CYP1A2}$, $f_{m\ CYP3A4}$, and $f_{m\ CYP2E1}$ of theophylline were estimated as 0.65, 0.20, and 0.15, respectively, and the $f_{m\ CYP1A2}$ and $f_{m\ CYP3A4}$ of lidocaine were estimated as 0.68 and 0.32, respectively.

3.2. Estimation of variability of $CL_{int,h,1A2}$

The WM and CV were calculated from information in the literature on the AUC/D and its variability for caffeine in healthy non-smokers (Table 2). The CV of the WM of AUC/D was calculated as 53.9% from the AUC/D and its CV values in 9 populations with a total of 96 subjects. Then, the CV of AUC/D was simulated using an arbitrary CV for $CL_{int,h,1A2}$ ranging from 0% to 100%. As a result, the optimal CV of $CL_{int,h,1A2}$ for the CV of the WM of AUC/D that had been collected from literature was estimated as 55% (Fig. 2).

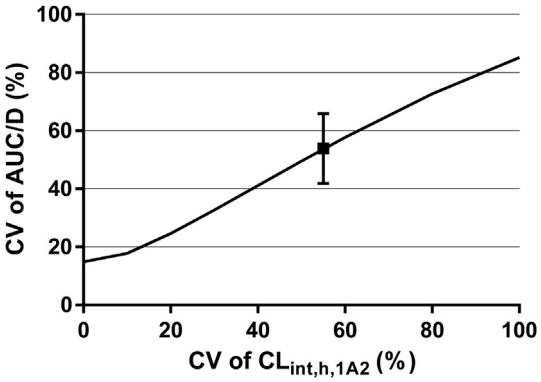

Fig. 2. Estimation of CV of $CL_{int,h,1A2}$ from caffeine data set. Closed diamond indicates observed CV of AUC/D of caffeine. Solid curve indicates the simulation curve of relationship between CV of $CL_{int,h}$ and CV of AUC/D with 2.5–97.5 percentile range. 1000 simulations were conducted in a virtual population with 96 subjects.

3.3. Prediction of variability of AUC/D for major CYP1A2 substrates

To confirm the accuracy of the predicted value for CV of $CL_{int,h,1A2}$ that was estimated from caffeine data, the CVs of AUC/D for the CYP1A2 substrates were predicted using the estimated CV of $CL_{int,h,1A2}$ of 55%. First, AUC/D and its CV for caffeine in each report were compared with the results simulated by setting the CV of

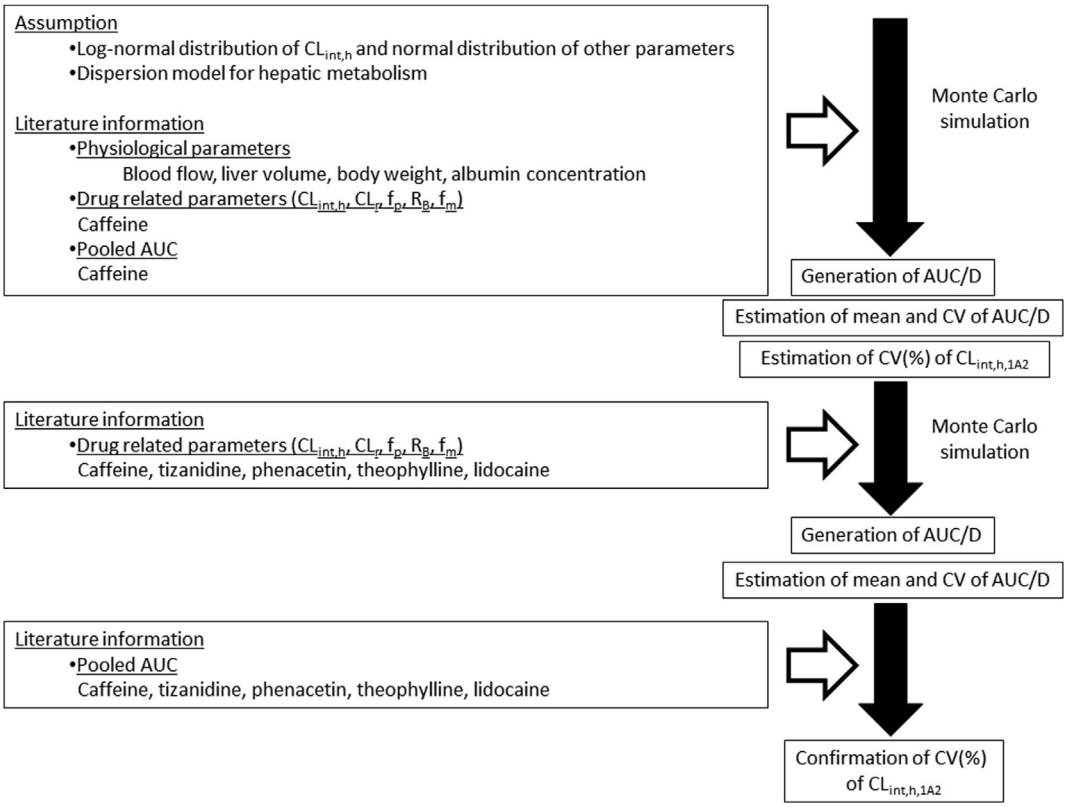

Fig. 1. Analytical scheme for estimation of the inter-individual variability.

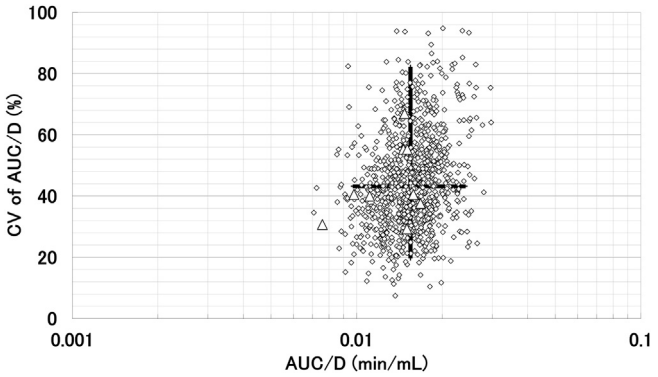

Fig. 3. Simulation of AUC variability of caffeine after oral administration. Open triangles indicate AUC/D and CV from literature. Open diamonds indicate simulated AUC/D and CV. Open circle indicates the mean AUC/D and CV of simulation with 2.5–97.5 percentile range. The range in the number of subjects in literature was from 6 to 16.

$CL_{int,h,1A2}$ at 55%. One thousand sets of AUC/D of caffeine were simulated in a virtual population of 6 subjects, which is the minimum number of subjects observed in the collected literature. As a result, most reported values (8 out of 9) were predicted within the 2.5–97.5 percentile range of simulated results (Fig. 3). Then, tizanidine and phenacetin were selected as major CYP1A2 substrates with $f_{m\ CYP1A2}$ over 0.9. The data for healthy non-smokers in 10 populations with a total of 167 subjects for tizanidine, and in 4 populations with a total of 61 subjects for phenacetin, were collected from literature. The CVs of the WM of AUC/D for tizanidine and phenacetin that were collected from literature were 86% and 80.2%, respectively (Table 2). AUC/D and its CV were simulated using a CV of $CL_{int,h,1A2}$ of 55% while changing $CL_{int,h,1A2}$. As a result, the simulated results (caffeine: CV; 53.6%, 2.5–97.5 percentile range; 44.5–68.5%, tizanidine: CV; 94.8%, 2.5–97.5 percentile range; 81.0–117.8%, phenacetin: CV; 66.1%, 2.5–97.5 percentile range; 53.4–91.3%) were comparable with the CVs of the WM of reported AUC/D for caffeine, tizanidine, and phenacetin (Fig. 4A). The simulation successfully predicted the reported variability in the AUC/D for CYP1A2 substrates. Moreover, results simulated using a 33% CV of $CL_{int,h,1A2}$ were clearly underestimated (Fig. 4A). Hence, the study supports the ability of this simulation to clearly distinguish the difference between the variability values of CYP1A2 and 3A4. Additionally, smaller AUC/D showed larger CV in simulation, and the same trend was observed in the reported AUC/D and CV for caffeine, tizanidine, and phenacetin. Moreover, the CV of the WM for each reported AUC/D for caffeine, tizanidine, and phenacetin was predicted by the Monte Carlo simulation using the CV of $CL_{int,h,1A2}$ at 55%. As a result, the CV of the WM of each reported AUC/D for caffeine, tizanidine, and phenacetin was successfully predicted within the 2.5–97.5 percentile range of simulation (Fig. 4A). As shown in Fig. 4A, high hepatic first-pass effect of high clearance drugs will make the inter-individual variability of the AUC/D for these drugs also high. In this study, inter-individual variability was accurately predicted for a wide range of low to high clearance drugs.

3.4. Prediction of variability of AUC/D for CYP1A2 substrates with contribution of other CYPs and renal clearance

The confirmation of prediction accuracy was conducted using theophylline and lidocaine which are CYP1A2 substrates with contribution of renal clearance and/or other CYPs. The data in 13 populations with a total of 158 subjects for theophylline, and in 6 populations with a total of 45 subjects for lidocaine, were collected from the literature on healthy non-smokers. CVs of the WM of AUC/D for theophylline and lidocaine collected from literature were 32.8% and 52.1%, respectively (Table 2).

To estimate the CV of $CL_{int,h,2E1}$, the AUC/D and its CV for chlorzoxazone, a CYP2E1 specific substrate, were collected from literature. The data in 5 populations with a total of 80 subjects for chlorzoxazone were collected from literature on healthy volunteers [39,42–45], and the WM of AUC/D and its CV were calculated as 3.93 min/l and 45.6%, respectively. Then, the CV of AUC/D was simulated using an arbitrary CV for $CL_{int,h,2E1}$ ranging from 0% to 100%. As a result, the optimal CV of $CL_{int,h,2E1}$ for the CV of the WM of AUC/D that had been collected from literature was estimated as 39% (Supplement Fig. 1). This CV was moderate compared with our reported CVs for other CYPs. Since various polymorphisms of CYP2E1 have been reported, it is possible that polymorphisms of CYP2E1 contribute to the CV of $CL_{int,h,2E1}$ [46,47]. Theophylline is

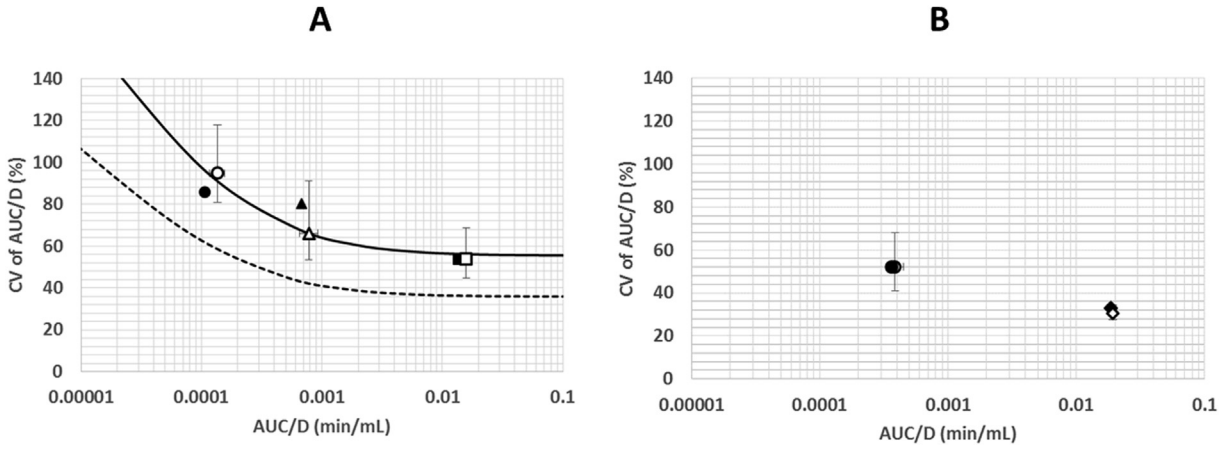

Fig. 4. Simulation of AUC variability of CYP1A2 substrates after oral administration. Graphs show (A) the major CYP1A2 substrates caffeine (squares), phenacetin (triangles), and tizanidine (circles) and (B) CYP1A2 substrates with contribution of other elimination, theophylline (diamonds) and lidocaine (circles). Closed symbols indicate observed AUC/D and CV. Open symbols indicate simulated AUC/D and CV with 2.5–97.5 percentile range. The solid and dotted curves indicate simulated AUC/D variability of 55% for $CL_{int,h,1A2}$ and 33% for $CL_{int,h,3A4}$ (previously reported). The contribution of elimination on theophylline is 83% in hepatic metabolism and 17% in urine excretion. The contribution ratio of CYPs in hepatic metabolism on theophylline is 65% by CYP1A2, 20% by CYP3A4, and 15% by CYP2E1. The contribution of elimination on lidocaine is 100% in hepatic metabolism, of which 68% is by CYP1A2 and 32% by CYP3A4.

partially metabolized by CYP2E1 in the liver. Therefore, a CV of $CL_{int,h,2E1}$ of 39% was used for the predictions for theophylline.

The CV of the WM of each reported AUC/D for theophylline and lidocaine was predicted by the Monte Carlo simulation using a CV of $CL_{int,h,1A2}$ of 55%. As a result, the CV of WM of each reported AUC/D for theophylline and lidocaine was successfully predicted within the 2.5–97.5 percentile range of simulation (theophylline: CV; 30.6%, 2.5–97.5 percentile range; 27.5–34.4%, lidocaine: CV; 52.3%, 2.5–97.5 percentile range; 41.0–67.9%) (Fig. 4B).

3.5. Prediction accuracy of variability of AUC/D for CYP1A2 substrates

To confirm the prediction accuracy, the reported CV of WM of AUC/D for 5 CYP1A2 substrates was compared to the simulated results (Fig. 5). Reported CV of WM of AUC/D for all substrates was successfully predicted within 80–120% of the simulated results. Hence, our methodology was confirmed to have good prediction accuracy.

4. Discussion

The purpose of this study is to evaluate a method for predicting the inter-individual variability of AUC/D for CYP1A2 substrates by setting the CV of $CL_{int,h,1A2}$ based on *in vivo* AUC/D data. In this study, since it was assumed that the amount of CYP enzyme was proportional to CL_{int}, the inter-individual variability of the amount of CYP enzyme was determined by that of CL_{int}. Therefore, the inter-individual variability of CL_{int} could be transferred from that of AUC and reflected the inter-individual variability of the amount of CYP enzyme. Once the inter-individual variability of the amount of each CYP enzyme is determined, the inter-individual variability of CL_{int} can be applied to CL_{int} of other substrates.

In this study, the top-down approach based on *in vivo* data was applied to predict the inter-individual variability of the AUC/D for CYP1A2 substrates. In contrast, the commercially available software SimCyp uses the bottom-up approach based on *in vitro* data to predict the inter-individual variability of pharmacokinetics [48]. The inter-individual variability in SimCyp was estimated using *in vitro* data on the amount of enzymes in HLM, and the estimated CV for CYP1A2 was 67%, which was larger than our result. Since previous reports show that the stability of HLM in *in vitro* studies can be strongly affected by freezing and thawing, as well as by storage conditions, we selected the top-down approach based on *in vivo* data [49,50].

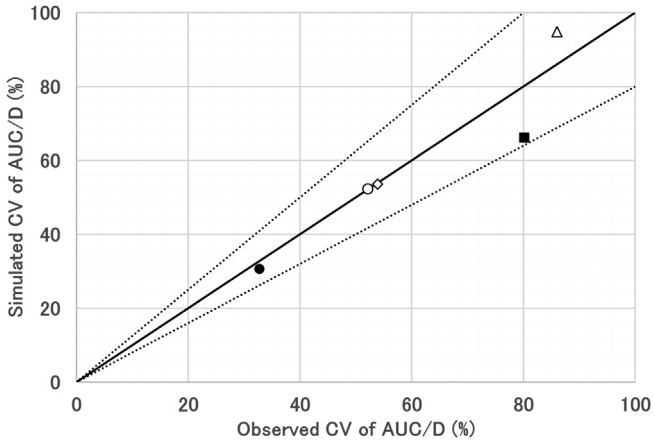

Fig. 5. Prediction accuracy of inter-individual variability of AUC/D in CYP1A2 substrates after oral administration. Observed data of caffeine (open diamond), phenacetin (closed square), tizanidine (open triangle), theophylline (closed circle), and lidocaine (open circle) are shown. The solid line indicates 100% accuracy and dotted lines indicate 120% and 80% accuracy.

CYP1A2, which is a major CYP in human liver that accounts for approximately 13% of total CYP activity in that organ [9], is involved in the metabolism of more than 100 drugs in human and is one of the key metabolizing enzymes involved in drug elimination [51]. Since over 15-fold [52] and 40-fold [53] inter-individual variability of CYP1A2 mRNA in human liver has been observed, there is a possibility that this inter-individual variability has a great impact on the drugs metabolized by CYP1A2. As described above, it has been reported that various genetic polymorphisms and environmental factors affect the activity of CYP1A2.

According to the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov), over 200 single nucleotide polymorphisms (SNPs) have been found in the CYP1A2 gene. Moreover, various variant alleles were also found. The variant alleles reported to have over 10% prevalence are *1A, *1B, *1C, *1D, *1F, *1L, and *1V. Although various genetic polymorphisms of CYP1A2 have been reported [15,16,54,55], there has been no report to indicate that genetic polymorphism has a clear impact on the pharmacokinetics of CYP1A2 substrates in healthy non-smokers; therefore, the effect of genetic polymorphism was not incorporated into this study.

It has also been reported that broccoli consumption [56], pan-fried meat consumption [57], and menstrual cycle phases [58] are environmental factors that possibly affect the activity of CYP1A2. As described above, since smoking has been reported as the most influential factor on the activity of CYP1A2, inter-individual variability in non-smokers was investigated in this study.

Since the $f_{m\ CYP1A2}$ of caffeine was almost 1 and caffeine showed a low hepatic first-pass effect, caffeine was selected to estimate the CV of $CL_{int,h,1A2}$. Because drugs with a low hepatic first-pass effect minimize the variability of that effect, they can be considered to estimate the CV of $CL_{int,h,1A2}$ more accurately. The inter-individual variability of other CYPs that have been reported by our group are 33% CV for CYP3A4 [6], 60% CV for CYP2D6 in EM [7], 66% CV for CYP2C19 in EM [59], and 18.1% CV for CYP2C9 in EM. The inter-individual variability of CYP1A2 (55%) was similar to that of CYP2D6 but otherwise relatively high among CYPs. As described above, since it has been reported that various genetic polymorphisms and environmental factors affect the activity of CYP1A2, it cannot be denied that these factors have a composite effect on the inter-individual variability of CYP1A2 in this study.

As shown in Fig. 4A, high clearance drugs showed high inter-individual variability of the AUC/D due to hepatic first-pass effect after oral administration. Kato et al. investigated the inter-individual variability of the AUC/D for CYP3A4 substrates after both intravenous and oral administration [6]. In the case of intravenous administration, since the hepatic clearance of a high clearance drug can be limited by hepatic blood flow, smaller inter-variability of the AUC/D for high clearance drugs was observed. In contrast, the inter-individual variability of the AUC/D after oral administration showed similar trend with our result in this study. These results suggest that the dosing route could be an important information to consider the inter-individual variability of the AUC/D.

As described above, high clearance drug shows high inter-variability of the AUC/D after oral administration. However, although lidocaine showed smaller AUC/D than phenacetin, lidocaine showed smaller inter-variability of the AUC/D than phenacetin. This could be due to the contribution of the inter-variability of $CL_{int,h,3A4}$ (33%) for lidocaine metabolism. Since the inter-variability of $CL_{int,h,3A4}$ (33%) was smaller than that of $CL_{int,h,1A2}$ (55%), the inter-variability of overall $CL_{int,h}$ for lidocaine should be smaller than 55%. Moreover, since no correlation was assumed between $CL_{int,h,3A4}$ and $CL_{int,h,1A2}$ in the Monte Carlo simulation, the standard

deviation of the sum of generated $CL_{int,h,3A4}$ and $CL_{int,h,1A2}$ should be smaller than the sum of each standard deviation. This is according to propagation of error. Therefore, the drugs with multiple metabolic pathways could show smaller inter-variability of AUC/D. The selection of the candidate drugs with multiple metabolic pathways can potentially reduce the inter-individual variability of AUC/D.

It is desirable that a clinical candidate drug has small inter-individual variability of pharmacokinetics for stable efficacy and safety. Since relatively high CV of $CL_{int,h}$ was estimated for CYP1A2, CYP2D6, and CYP2C19, drugs that are metabolized by these CYPs will require a wide-ranging efficacy concentration and a broad safety margin in clinical development. Also, as shown in Fig. 4A, since high clearance drugs are expected to have large inter-individual variability of AUC/D caused by the hepatic first-pass effect, selecting candidate drugs with low clearance is one approach to reducing the inter-individual variability of AUC/D in human.

5. Conclusions

In this study, we propose a methodology that can predict the inter-individual variability of AUC for CYP1A2 substrates in human. Since the estimated 55% CV of $CL_{int,h,1A2}$ is relatively high among CYPs, it can be considered that prediction of inter-individual variability of AUC for CYP1A2 substrates in human will strongly contribute to improving the success rate and shortening the length of clinical trials. In the future, inter-individual variability of pharmacokinetics in human will be comprehensively predictable by this method of estimating the inter-individual variability, for not only for CYPs, but also for transporters in the liver and kidney, and not only in healthy volunteers but also in children, the elderly, and in populations with a specific disorder. Also, this methodology of predicting inter-individual variability of pharmacokinetics can be expanded to predict the inter-individual variability of efficacy and safety for various drugs. Moreover, we consider it will contribute to efficient clinical development and reduction of unexpected side effect.

Conflict of interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dmpk.2016.03.003.

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