Simulation for Clinical Repeated-Dose Pharmacokinetic Trials Applying a Peak-and-Trough Sampling Design to Estimate Oral Clearance

Kazuya ISHIDA, Yuichiro KAYANO, Masato TAGUCHI, and Yukiya HASHIMOTO*

Graduate School of Pharmaceutical Sciences, University of Toyama; 2630 Sugitani, Toyama 930-0194, Japan.

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We performed a simulation for the clinical pharmacokinetic study, in which blood was sampled at two time points corresponding to the peak concentration (C_{peak}) and trough concentration (C_{trough}) following repetitive oral drug administration to subjects. We estimated the approximate oral clearance (CL/F_{approx}) as $2 \cdot D / (C_{\text{peak}} \cdot \tau + C_{\text{trough}} \cdot \tau)$, where D is the dose, and τ is the dosing interval. The CL/F_{approx} value was accurate for drugs with a long-elimination half-life, and the estimation error of the CL/F value was slightly increased for drugs with a shorter elimination half-life. The accuracy of CL/F_{approx} in each subject was not affected by the magnitude of the interindividual pharmacokinetic variability, but was significantly decreased by the larger measurement error of drug concentrations (or intraindividual pharmacokinetic variability). We further performed several computer simulations to mimic statistical hypothesis testing following the clinical repeated-dose pharmacokinetic trials. The statistical power to detect the difference of oral clearance between two groups was marginally dependent on the measurement error of drug concentration, but was highly dependent on the interindividual pharmacokinetic variability. These findings suggested that the peak-and-trough sampling design to estimate the CL/F_{approx} value is useful for clinical repeated-dose pharmacokinetic trials, and that the study design and protocol should be evaluated carefully by computer simulation prior to a real clinical trial.

Key words clinical pharmacokinetic trial; oral clearance; sampling design; steady-state simulation

The clinical pharmacologists are charged with the obligation to quantify the dose–response relationship (pharmacokinetics and pharmacodynamics) of clinically useful drugs.¹⁾ Clinical pharmacokinetic studies are performed to determine the appropriate use of medicines according to patient characteristics, such as the disease and genotype of drug-metabolizing enzymes, and to predict the influence of pharmacokinetic drug interactions.^{2–7)} The area under the concentration–time curve (AUC) and/or oral clearance (CL/F) of orally administered drugs is a key pharmacokinetic parameter to evaluate drug exposure in the patient population. In the present study, we evaluated the usefulness of clinical repeated-dose pharmacokinetic trials, applying a simple peak-and-trough sampling design in order to estimate the CL/F value. That is, blood was sampled at two points corresponding to the peak concentration (C_{peak}) and trough concentration (C_{trough}) following repetitive oral drug administration to a patient (Fig. 1). The approximate CL/F (CL/F_{approx}) value in the patient

may be estimated by the following equations:

$$AUC \approx \left(\frac{C_{\text{peak}} + C_{\text{trough}}}{2} \right) \cdot \tau \quad (1)$$

$$CL/F_{\text{approx}} = \frac{D}{AUC} = \frac{2 \cdot D / \tau}{C_{\text{peak}} + C_{\text{trough}}} \quad (2)$$

where D is the dose, and τ is the dosing interval. The specific purpose of the present study was to investigate the accuracy of the CL/F_{approx} value estimated by the peak-and-trough sampling design. We further performed several computer simulations to mimic statistical hypothesis testing following a clinical practice-resembling repeated-dose pharmacokinetic trial.

MATERIALS AND METHODS

Evaluation of Accuracy of CL/F_{approx} Estimated by C_{peak} and C_{trough} To evaluate the accuracy of the CL/F_{approx} value for various hypothetical drugs, we assumed a 1-compartment model with first-order input, and calculated the ratio of CL/F_{approx} to true oral clearance (CL/F) as follows:

$$\begin{aligned} \frac{CL/F_{\text{approx}}}{CL/F} &= \frac{2 \cdot D / \tau}{k_e \cdot V / F} \cdot (C_{\text{peak}} + C_{\text{trough}})^{-1} \\ &= \frac{2 \cdot (k_a - k_e)}{k_e \cdot k_a \cdot \tau} \cdot \left\{ \frac{\exp(-k_e \cdot t_{\text{max}}) + \exp(-k_e \cdot \tau)}{1 - \exp(-k_e \cdot \tau)} \right. \\ &\quad \left. - \frac{\exp(-k_a \cdot t_{\text{max}}) + \exp(-k_a \cdot \tau)}{1 - \exp(-k_a \cdot \tau)} \right\}^{-1} \end{aligned} \quad (3)$$

where V/F is the apparent volume of distribution, k_e is the elimination rate constant, k_a is the absorption rate constant, and t_{max} is the time to reach maximal drug concentration after the last dose, described as follows:

Fig. 1. Approximate Estimation of AUC by Measuring C_{peak} and C_{trough} Following Clinical Practice-Resembling Repetitive Dosing

* To whom correspondence should be addressed. e-mail: yukiya@pha.u-toyama.ac.jp

Table 1. Accuracy of CL/F_{approx} for Hypothetical Drugs with Various k_e and k_a

$t_{1/2,k_e}$ ^{a)} (h)	$t_{1/2,k_a}$ ^{b)} (h)	τ (h)	t_{max} (h)	C_{peak}	CL/F_{approx}
				C_{trough}	CL/F
48	1/3	12	1.517	1.155	1.009
48	2/3	12	2.379	1.133	1.017
48	1	12	2.996	1.115	1.020
48	2	12	4.082	1.078	1.020
48	3	12	4.612	1.056	1.016
24	1/3	12	1.487	1.336	1.009
24	2/3	12	2.331	1.285	1.017
24	1	12	2.936	1.245	1.020
24	2	12	4.006	1.162	1.020
24	3	12	4.531	1.116	1.016
12	1/3	12	1.430	1.790	1.005
12	2/3	12	2.238	1.660	1.025
12	1	12	2.821	1.558	1.034
12	2	12	3.859	1.355	1.037
12	3	12	4.373	1.248	1.031
8	1/3	12	1.376	2.405	0.990
8	2/3	12	2.150	2.152	1.023
8	1	12	2.710	1.957	1.041
8	2	12	3.716	1.583	1.052 ^{c)}
8	3	12	4.218	1.397	1.044
4	1/3	12	1.234	5.919	0.892 ^{c)}
4	2/3	12	1.914	4.783	0.970
4	1	12	2.411	3.957	1.020
4	2	12	3.321	2.530	1.072 ^{c)}
4	3	12	3.786	1.945	1.071 ^{c)}

a) $t_{1/2,k_e}=0.693/k_e$. b) $t_{1/2,k_a}=0.693/k_a$. c) Errors are more than 5%.

$$t_{\text{max}} = \frac{1}{k_a - k_e} \cdot \ln \left\{ \frac{k_a}{k_e} \cdot \frac{1 - \exp(-k_e \cdot \tau)}{1 - \exp(-k_a \cdot \tau)} \right\} \quad (4)$$

Table 1 summarizes the $t_{1/2,k_e}$ ($=0.693/k_e$), $t_{1/2,k_a}$ ($=0.693/k_a$), τ , and t_{max} values of various hypothetical drugs evaluated in the present study.

We further evaluated the effect of the measurement error of C_{peak} and C_{trough} (or intraindividual pharmacokinetic variability) on the accuracy of the CL/F_{approx} value. It is possible that interindividual pharmacokinetic variability is also responsible for the measurement error of the C_{peak} , because we do not know the true t_{max} in a subject before blood sampling. Therefore, we assumed typical hypothetical drugs with a mean $t_{1/2,k_e}$ of 1 h, CL/F of 1, V/F of 12/0.693, $t_{1/2,k_a}$ of 12 h, and t_{max} of 2.821 h (Table 1), and with various inter- and intraindividual pharmacokinetic variability. The k_a value of a drug in the i -th individual (k_{ai}) is described as follows:

$$k_{ai} = 0.693 \cdot \exp(\eta_{k_{ai}}) \quad (5)$$

where $\eta_{k_{ai}}$ is a random variable distributed normally with a mean of zero and variance of $\omega_{k_a}^2$. Similarly, the k_e value of the drug in the i -th individual (k_{ei}) is described as follows:

$$k_{ei} = \frac{CL/F_i}{V/F_i} \quad (6)$$

$$CL/F_i = 1 \cdot \exp(\eta_{CL/F_i}) \quad (7)$$

$$V/F_i = \frac{12}{0.693} \cdot \exp(\eta_{V/F_i}) \quad (8)$$

where CL/F_i is oral clearance in the i -th individual, and η_{CL/F_i} is the random variable distributed normally with a mean of

Table 2. The ω and σ Values of Hypothetical Drugs²⁻⁸⁾

	A	B	C	D	E	F
$\omega_{CL/F}$	0.4	0.4	0.4	0.4	0.3	0.5
$\omega_{V/F}$	0.4	0.4	0.4	0.4	0.3	0.5
ω_{k_a}	0.8	0.8	0.8	0.8	0.6	1.0
σ_3	0.05	0.1	0.15	0.2	0.1	0.1
σ_{12}	0.05	0.1	0.15	0.2	0.1	0.1

Table 3. Protocols and Results of Various In-Silico Clinical Trials

Trial	n	CL/F	$\omega, \sigma^a)$	Statistical significance		
				N.S. ^{b)}	$p < 0.05$	$p < 0.01$
1	20	1.0	B	6/30	10/30	14/30
	20	0.7	B			
2	20	1.0	C	10/30	11/30	9/30
	20	0.7	C			
3	20	1.0	D	11/30	9/30	10/30
	20	0.7	D			
4	20	1.0	E	2/30	2/30	26/30
	20	0.7	E			
5	20	1.0	F	13/30	8/30	9/30
	20	0.7	F			
6	30	1.0	B	13/30	10/30	7/30
	10	0.7	B			
7	10	1.3	B			
	20	1.0	B	5/30	6/30	19/30
	10	0.7	B			

a) See Table 2. b) Not significant.

zero and variance of $\omega_{CL/F}^2$. V/F_i is the apparent volume of distribution in the i -th individual, and η_{V/F_i} is the random variable distributed normally with a mean of zero and variance of $\omega_{V/F}^2$. We assumed that the sampling time is 3 h and 12 h after the twice-daily dose ($\tau=12$ h) for all subjects. Thus, drug concentrations at 3 h (C_{3i}) and 12 h (C_{12i}) after the last dose in the i -th individual are described as follows:

$$C_{3i} = \frac{D_i \cdot k_{ai}}{V/F_i \cdot (k_{ai} - k_{ei})} \cdot \left\{ \frac{\exp(-k_{ei} \cdot 3)}{1 - \exp(-k_{ei} \cdot 12)} - \frac{\exp(-k_{ai} \cdot 3)}{1 - \exp(-k_{ai} \cdot 12)} \right\} \cdot \exp(\varepsilon_{3i}) \quad (9)$$

$$C_{12i} = \frac{D_i \cdot k_{ai}}{V/F_i \cdot (k_{ai} - k_{ei})} \cdot \left\{ \frac{\exp(-k_{ei} \cdot 12)}{1 - \exp(-k_{ei} \cdot 12)} - \frac{\exp(-k_{ai} \cdot 12)}{1 - \exp(-k_{ai} \cdot 12)} \right\} \cdot \exp(\varepsilon_{12i}) \quad (10)$$

where D_i is the dose in the i -th individual, and ε_{3i} and ε_{12i} are the random variables distributed normally with means of zero and variance of σ_3^2 and σ_{12}^2 , respectively. Finally, CL/F_{approx} in the i -th individual ($CL/F_{\text{approx},i}$) is described as follows:

$$CL/F_{\text{approx},i} = \frac{2 \cdot D_i / 12}{C_{3i} + C_{12i}} \quad (11)$$

The accuracy of $CL/F_{\text{approx},i}$ was evaluated by examining the correlation between CL/F_i and $CL/F_{\text{approx},i}$ in 50 subjects. Table 2 summarizes the ω and σ values of various hypothetical drugs evaluated in the present study.²⁻⁸⁾

Simulation for Statistical Hypothesis Testing We further performed several computer simulations to mimic statis-

tical hypothesis testing following the clinical repeated-dose pharmacokinetic trial. We assumed a hypothetical drug with a mean $t_{1/2,k_a}$ of 1 h and V/F of 12/0.693. The other characteristics of the test drug, the number of subject groups, and the number of subjects in each group (n) are summarized in Table 3. $CL/F_{\text{approx},i}$ in each subject was calculated as described above, and the difference in the mean oral clearance between two groups was evaluated by Student's t -test. If the number of groups was three, we used one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

We first evaluated the accuracy of CL/F_{approx} for hypothetical drugs with various k_a and k_e (Table 1). In the present study, $t_{1/2,k_a}$ values ($0.693/k_a$) were assumed to range from 1/3 h to 3 h, and τ was fixed at 12 h for all hypothetical drugs. The t_{max} values ranged from 1.517 to 4.612 h for drugs with $t_{1/2,k_e}$ ($0.693/k_e$) of 48 h, and the ratios of CL/F_{approx} to CL/F were almost identical to 1, indicating that CL/F_{approx} values were estimated very accurately (Table 1). In addition, it is apparently conceivable that C_{peak} may be close to C_{trough} for drugs with long $t_{1/2,k_e}$ relative to τ , and that the AUC value for

the drug may be nearly equal to $C_{\text{trough}} \cdot \tau$. However, the $C_{\text{peak}}/C_{\text{trough}}$ values were not always nearly equal to 1 even in the drug with $t_{1/2,k_e}$ of 48 h (Table 1). Therefore, the peak-and-trough sampling design is preferable to the single-trough sampling design especially for the drug with short $t_{1/2,k_a}$. Furthermore, the accuracy of CL/F_{approx} was also fairly good for drugs with $t_{1/2,k_e}$ values of 24 h, 12 h, and 8 h (Table 1). On the other hand, the ratios of CL/F_{approx} to CL/F ranged from 0.892 to 1.072 for the drugs with $t_{1/2,k_e}$ of 4 h. It should be noted that we should not use CL/F_{approx} without extreme caution for drugs with very short $t_{1/2,k_e}$ relative to τ .

We next evaluated the accuracy of CL/F_{approx} for hypothetical drugs with various ω and σ values. We assumed drugs with a mean $t_{1/2,k_a}$ of 1 h, CL/F of 1, V/F of 12/0.693, $t_{1/2,k_e}$ of 12 h, and t_{max} of 2.821 h in the present study. The sampling time was assumed to be 3 h and 12 h after a repetitive twice-daily dose ($\tau=12$ h) for all 50 subjects, because we could not know the true t_{max} in individual subjects. Figure 2A shows the correlation between $CL/F_{\text{approx},i}$ and CL/F_i for a drug with $\omega_{CL/F} = \omega_{V/F} = 0.4$, $\omega_{k_a} = 0.8$, and $\sigma_3 = \sigma_{12} = 0.05$. Considerably large interindividual variability was assumed for k_a , CL/F_i , and V/F_i ; however, the accuracy of $CL/F_{\text{approx},i}$ was fairly good in all 50 subjects (Fig. 2A). Figure 3 shows the simulated blood drug concentration–time curve in 6 typical subjects, who are depicted with closed circles in Fig. 2A. We as-

Fig. 2. Accuracy of $CL/F_{\text{approx},i}$ for Hypothetical Drugs with Various ω and σ

The ω and σ values for A–F are shown in Table 2. Closed circles in A indicate 6 typical subjects whose blood drug concentrations are shown in Fig. 3.

Fig. 3. Simulated Blood Drug Concentration–Time Curves in 6 Typical Subjects Shown in Fig. 2A

Open circles represent the measured blood concentration.

sumed that we measured C_{3i} instead of C_{peak} in each subject; nevertheless, C_{3i} seemed to be a good estimate of C_{peak} in each subject (Fig. 3). In addition, $CL/F_{\text{approx},i}$ was nearly equal to CL/F_i even in the subject with considerably short $t_{1/2,k_e}$ (ID=32). These findings suggested that the accuracy of $CL/F_{\text{approx},i}$ is fairly good even for drugs with large ω values when the measurement error of drug concentration (or intraindividual pharmacokinetic variability) is small.

It is readily conceivable that the large measurement error of drug concentration deteriorates the accuracy of $CL/F_{\text{approx},i}$ significantly. The estimation error of $CL/F_{\text{approx},i}$ was only marginally increased for a drug with $\sigma_3 = \sigma_{12} = 0.1$ as compared with a drug with $\sigma_3 = \sigma_{12} = 0.05$ (Figs. 2A, B), whereas it was considerably large for drugs with $\sigma_3 = \sigma_{12} = 0.15$ and $\sigma_3 = \sigma_{12} = 0.2$ (Figs. 2C, D). It should be noted that we may not estimate the $CL/F_{\text{approx},i}$ value for drugs with large σ values without multiple drug assays and/or blood sampling to obtain reliable mean values of C_{peak} and C_{trough} , although it is still unclear whether the σ value of 0.2 is realistic.⁸⁾ In addition, the further decrease/increase in ω values seemed not to induce significant alterations of the accuracy of $CL/F_{\text{approx},i}$ (Figs. 2E, F).

We performed additional computer simulations to mimic statistical hypothesis testing following clinical repeated-dose pharmacokinetic trials. The hypothetical drugs, dosing regimen, and sampling design were the same as those shown in Figs. 2 and 3. In addition, the CL/F value of the drug was assumed to be decreased to 0.7 by another concomitantly administered drug, or to be altered by the polymorphisms of drug-metabolizing enzymes. Table 3 shows the protocols and results of various in-silico clinical pharmacokinetic trials, where the number of replications of each trial was 30 in the present study. The statistical power to detect the difference of oral clearance between two groups was considerably high for a drug with $\omega_{CL/F} = \omega_{V/F} = 0.4$, $\omega_{k_a} = 0.8$, and $\sigma_3 = \sigma_{12} = 0.1$, when the number of subjects in each group was set to 20 (Trial 1 in Table 3). The statistical power can be dependent on the magnitude of the measurement error of drug concentration because of the subsequent estimation error of $CL/F_{\text{approx},i}$. Indeed, statistical significance following the t -test was marginally decreased for drugs with $\sigma_3 = \sigma_{12} = 0.15$ and $\sigma_3 = \sigma_{12} = 0.2$ (Trials 2, 3). On the other hand, the interindividual pharmacokinetic variability of drugs significantly affected the statistical power to detect the difference of oral

clearance between two groups (Trials 4, 5). An unbalanced number of subjects in each group markedly affected the statistical significance (Trials 6, 7). This simple experimental strategy may also be useful for designing the protocol of real clinical pharmacokinetic trials.

It should be noted that we did not extend the simulation to systematic evaluation of accuracy of CL/F_{approx} for drugs with absorption lag time and/or with biexponential disposition in the present study, because accuracy of the estimated CL/F value should be dependent not only on the pharmacokinetic characteristics of drugs (mean pharmacokinetic parameters and inter- and intraindividual variability), but also on study protocols/designs (τ and sampling time). Eventually, the accuracy and precision of the analysis method to estimate oral clearance (CL/F_{approx}) for a target drug of individual researchers should be evaluated carefully prior to a real clinical trial.

In conclusion, the present simulation study suggested that the peak-and-trough sampling design to estimate the CL/F_{approx} value is useful for clinical repeated-dose pharmacokinetic trials, and that the study design and protocol should be evaluated carefully by computer simulation prior to a real clinical trial.

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