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Bottom-up modeling and simulation of tacrolimus clearance: prospective investigation of blood cell distribution, sex and CYP3A5 expression as covariates and assessment of study power

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ABSTRACT: The objectives were to investigate the ability of population-based *in vitro–in vivo* extrapolation (IVIVE) to reproduce the influence of haematocrit on the clearance of tacrolimus, observed previously, and to assess the power of clinical studies to detect the effects of covariates on the clearance of tacrolimus.

A population-based pharmacokinetic simulator (Simcyp) was used to simulate tacrolimus clearance from *in vitro* metabolism data and demographic characteristics of Japanese liver transplant patients (JLTs). The relationship between haematocrit and dose-to-concentration (D/C) ratio was validated using seven JLTs, whose highly variable haematocrit and D/C ratio were previously analysed. This validation was used as a surrogate for establishing 'interindividual' variability and to assess the power of clinical studies to discern the effect of haematocrit, sex and CYP3A5 genotype on tacrolimus clearance in a virtual JLT population.

The relationship between haematocrit and D/C ratio was reproducible by Simcyp and corresponded well to those observed in seven JLTs. The number of JLTs required to detect the influence of CYP3A5 genotype and sex were estimated to be about 50 and > 600, respectively, which was consistent with the results of previous population pharmacokinetic studies for tacrolimus.

In conclusion, population-based IVIVE is considered to be a useful approach to assess the influence of covariates *a priori* before conducting clinical studies. This is also helpful with study design and assessment of the statistical power of clinical studies involving population-based pharmacokinetics to detect the effects of covariates. Copyright © 2011 John Wiley & Sons, Ltd.

Key words: haematocrit; covariate analysis; population pharmacometrics; *in vitro in vivo* extrapolation (IVIVE); modeling and simulation

Introduction

Tacrolimus, a potent immunosuppressant, is used widely in patients after organ transplant. Considerable intra- and inter-individual variability has been shown in the pharmacokinetics of tacrolimus.

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Analysis of observed data using non-linear mixed effect modeling (a 'top-down approach') has proved useful in evaluating the influence of covariates on the pharmacokinetics of many drugs. Indeed, a series of population pharmacokinetic (POPPK) studies have been carried out in liver transplant patients quantitatively to investigate the effects of covariates such as total bilirubin, postoperative days, cytochrome P450 (CYP) 3A5 genotype, MDR1 (ABCB1) genotype, blood biochemistry (AST, ALT, gamma GTP etc.) and haematocrit on tacrolimus [1–9]. It was previously reported that haematocrit is one of the factors for not only inter-individual but also intraindividual variations of the ratio of 'dose/trough concentrations' of tacrolimus (D/C) in plasma (D/C_p) and whole blood (D/C_{WB}) in Japanese liver transplant patients (JLTs) [10]. These results, together with POPPK studies, enable us to individualize the dosage regimen of tacrolimus using Bayesian estimation. Whilst these classical studies require clinically observed data to discern inferences between certain subgroups and to identify covariates, the alternative use of mechanistic physiologically based pharmacokinetic (PBPK) modeling (a 'bottom-up approach') may highlight such differences *a priori*. However, to our knowledge, this approach has not been tested for its ability to recover the observed effects of covariates on the pharmacokinetics of tacrolimus in liver transplant patients. PBPK combined with IVIVE simulations could be valuable in assessing the power of prospective studies aimed to detect covariate effects. Examples of these are related to design of POPPK studies for DDI [11] or the impact of pharmacogenetics [12,13] and optimal design for such studies.

With respect to tacrolimus and covariates of its kinetics, although some researchers have shown a sex difference in the hepatic abundance of CYP3A4 [14], none of the seven reported POPPK studies [1–9] detected significant sex differences and thus this covariate was not included in the final model. On the other hand, two POPPK studies investigated the effects of the CYP3A5 genotype and both succeeded in detecting a significant effect, resulting in inclusion of the CYP3A5 genotype in their final covariate model. It was shown recently that these differences may be explained by the difference in the statistical power of detection [15].

The aims of the present study were: (1) to investigate the ability to reproduce the effects of haematocrit on D/C of tacrolimus observed in our previous study [10] using a mechanistic model of clearance implemented in a population-based PBPK simulator (Simcyp®, www.simcyp.com) ('SIMULATION study'), and (2) to assess the statistical power of *in vivo* studies using a virtual JLT population to detect the effects of two other covariates, CYP3A5 genotype and sex, on D/C_p or D/C_{WB} of tacrolimus ('POWER study').

Methods

Patients

Demographic and physiological data from seven JLT patients were adopted from our previous report [10] and summarized in Table 1. Blood samples from the start of oral administration to 70 postoperative days (POD) were used for the analysis. Patient 4 had been excluded from our previous report due to the 'extraordinary' high D/C value [10] (potentially different genotype for CYP3A5).

Tacrolimus kinetics

In vitro tacrolimus metabolism data were taken from a study using recombinant CYP3A4 and 3A5 [16]. Data were corrected for differences in activity between the recombinant enzyme and native human liver enzyme using an Inter System Extrapolation Factor (ISEF) of 0.34, which is specific to the BD Gentest baculovirus system [17]. The V_{max} and K_m values for 13-O-desmethylation were 2.72 [nmol/min/nmol CYP] and 0.21 [μM] for CYP3A4, and 5.78 [nmol/min/nmol CYP] and 0.21 [μM] for CYP3A5, respectively. The V_{max} and K_m values for 12-hydroxylation were 0.204 [nmol/min/nmol CYP] and 0.29 [μM] for CYP3A4, and 0.476 [nmol/min/nmol CYP] and 0.35 [μM] for CYP3A5, respectively. The plasma unbound fraction of tacrolimus was set to 0.013 [18]. The blood-to-plasma concentration ratio of tacrolimus was assumed to be 32.0 at a haematocrit value of 40% [10].

In vitro–In vivo Extrapolation

A population-based pharmacokinetic simulator (Simcyp Ver 7.11) developed by Simcyp® Ltd

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Table 1. Demographic data, observation period and dose of seven subjects

No.	Age	Body weight (POD 30)(kg)	Estimated height (cm)	Sex	Observation period (POD, day)		Number of blood samples	Dose (mg/12 h)
					From	To		
1	60	55	163.8	M	16	70	106	1.861
2	46	48	167.6	M	14	70	49	2.625
3	50	40	154.2	F	15	70	52	1.798
4	51	46	153.9	F	9	70	60	4.099
5	61	40	151.0	F	27	70	28	2.107
6	30	48	157.2	F	13	70	56	1.278
7	53	45	153.4	F	20	70	52	1.946

No.	Haematocrit	
	Geometric Mean	CV (%)
1	0.298	18.24%
2	0.281	17.18%
3	0.238	18.78%
4	0.322	14.62%
5	0.280	8.63%
6	0.280	6.77%
7	0.228	9.99%

Patient 4 had been excluded from the figure in our previous report [8] due to extraordinarily high D/C value.

(http://www.simcyp.com) was used to simulate the oral tacrolimus clearance and D/C_{p} and D/C_{WB} values in each patient with respective haematocrit values. As the purpose of the simulation was to calculate the oral clearance in respect to the change in haematocrit, the study did not simulate the time-profile of tacrolimus using a full PBPK model, in which distribution parameters are required to be taken into consideration, instead a time and concentration independent (static) model was applied. A step-by-step description of this *in vitro–in vivo* extrapolation (IVIVE) strategy using the Simcyp® population-based simulator has been reported previously [19].

Defining virtual subjects (SIMULATION study)

To simulate tacrolimus clearance over an extended observation period in each subject, seven 'population' datasets, corresponding to the demographic details of each patient, was prepared using Simcyp®. In other words, Monte Carlo simulation was used in these seven Japanese adult liver transplant 'populations' to create intraindividual variability as opposed to its common use for simulating interindividual variability.

The intraindividual variation in various elements of IVIVE was assumed to be 2% unless otherwise specified. Actual data for individual subjects such as dose (mean ± CV), age, body weight (actual value in postoperative day 30 with CV of 2%) and haematocrit (mean ± CV) were incorporated as 'population' parameters. As height was not recorded in our previous study [10], it was estimated from body weight and age using information on the Japanese population. Liver volume was assumed to be 88% of normal Japanese subjects [20], because, after transplantation, liver volume is known to almost double in 1 week, and reaches a steady level (88% of normal) from 1 to 3 months after transplantation [21,22]. The abundances of CYP3A4 and CYP3A5 protein per milligram of microsomal protein in gut and liver were assumed to be equal to those in normal Japanese subjects, while their CV value was assumed to be 9.75%, based on the intraindividual variation of midazolam clearance [23]. Patients 1–3, 5–7 were assumed to be CYP3A5 poor metabolizers (PM) and patient 4, who had a considerably higher D/C ratio, was assumed to be an extensive metabolizer (EM).

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Virtual population ('POWER study')

A dataset representative of a Japanese adult liver transplant population was also created to assess the statistical power to detect the effects of covariates. The age range was set from 20 to 61 years because the oldest subject in our previous report [10] was 61 years old. Heights for male and female were calculated by Simcyp® using Equation (1) with *C0*, *C1* and *C2* values obtained from Japanese population data [20].

$$\text{Mean height} = C0 + C1 \cdot \text{Age} + C2 \cdot \text{Age}^2 \quad (1)$$

where *C0*, *C1* and *C2* were 168.49, 0.174 and −0.0042 for male and 155.06, 0.207 and −0.0045 for female, respectively. The CV value was set at 3.54% for both male and female.

Body weights for male and female were calculated by the following Equation (2):

$$\text{Body weight} = \exp\left(C0 + C1 \cdot \text{Height}\right) \quad (2)$$

where *C0* and *C1* were 3.091 and 0.005 for male and 3.371 and 0.003 for female, respectively, with CV values of 14.6% and 16.6% for male and female, respectively.

The liver volume was again assumed to be 88% of normal Japanese subjects. Haematocrit and its variability were taken from the observed data of our previous study [10]; geometric mean and CV values were 0.292 and 18.13% for male and 0.277 and 17.95% for female, respectively.

The tacrolimus dose was assumed to be 2.245 mg every 12 h, the mean dose in our previous study [10]. The ratio of male: female individuals in the population were 1:2 based on the sex distribution of liver transplant patients in Japan [24].

The statistical power to detect the effects of CYP3A5 genotype and sex was determined from 20 trials by the following protocol using a virtual Japanese liver transplant population defined above with modification as follows.

In the study to assess the statistical power to detect differences due to sex, hepatic CYP3A4 abundance in female Japanese was assumed to be 45.3% higher than that in male Japanese, based on the abundance ratio in male and female Caucasians, and the population PM frequency for CYP3A5 was assumed to be 58% [25]. Steady-state concentrations of tacrolimus in blood and plasma were simulated in individual Japanese virtual liver transplant subjects in studies of different sizes ($n = 6$–600).

In order to investigate the effect of CYP3A5 genotype, no sex difference in CYP3A4 abundance was assumed. Studies containing 4–500 virtual subjects from the same population with an EM:PM ratio of 1:1 were simulated.

The power of the study was measured by the rate of detection of statistically significant differences in D/C ratio by means of Student's *t*-test with a value of $p < 0.05$.

Results

Simulation of the effects of haematocrit on D/C of tacrolimus

Figure 1 shows the simulated relationship between haematocrit and D/C (D/C_p and D/C_{WB}) along with the relationship observed *in vivo*. The simulated relationships corresponded well to those observed. The difference between simulated D/C_p or D/C_{WB} value and observed values for each patient was within two-fold. Moreover, the correlation coefficient between D/C_{WB} ratio and haematocrit was larger than that between D/C_p ratio and haematocrit ($r = -0.51$ vs -0.20), which is also consistent with the observed results ($r = -0.53$ vs $r = -0.23$). The unusually high D/C values observed for one patient (patient 4) could be explained, at least in part, by the CYP3A5 EM genotype although the observed D/C value was higher than the simulated value the difference was less than two-fold.

Statistical power to detect the effects of CYP3A5 genotype and sex

For the detection of hypothetical sex difference assuming a 45.3% higher abundance of hepatic CYP3A4 in female than in males, 600 or more subjects were required to detect statistically significant differences in the tacrolimus D/C value between male and female individuals ($p < 0.05$; two tail *t*-test) with 90% probability (Figure 2A). On the other hand, only about 50 subjects were required to detect statistically significant differences in the tacrolimus D/C value among CYP3A5 EMs and PMs with 90% probability (Figure 2B). No difference in statistical power

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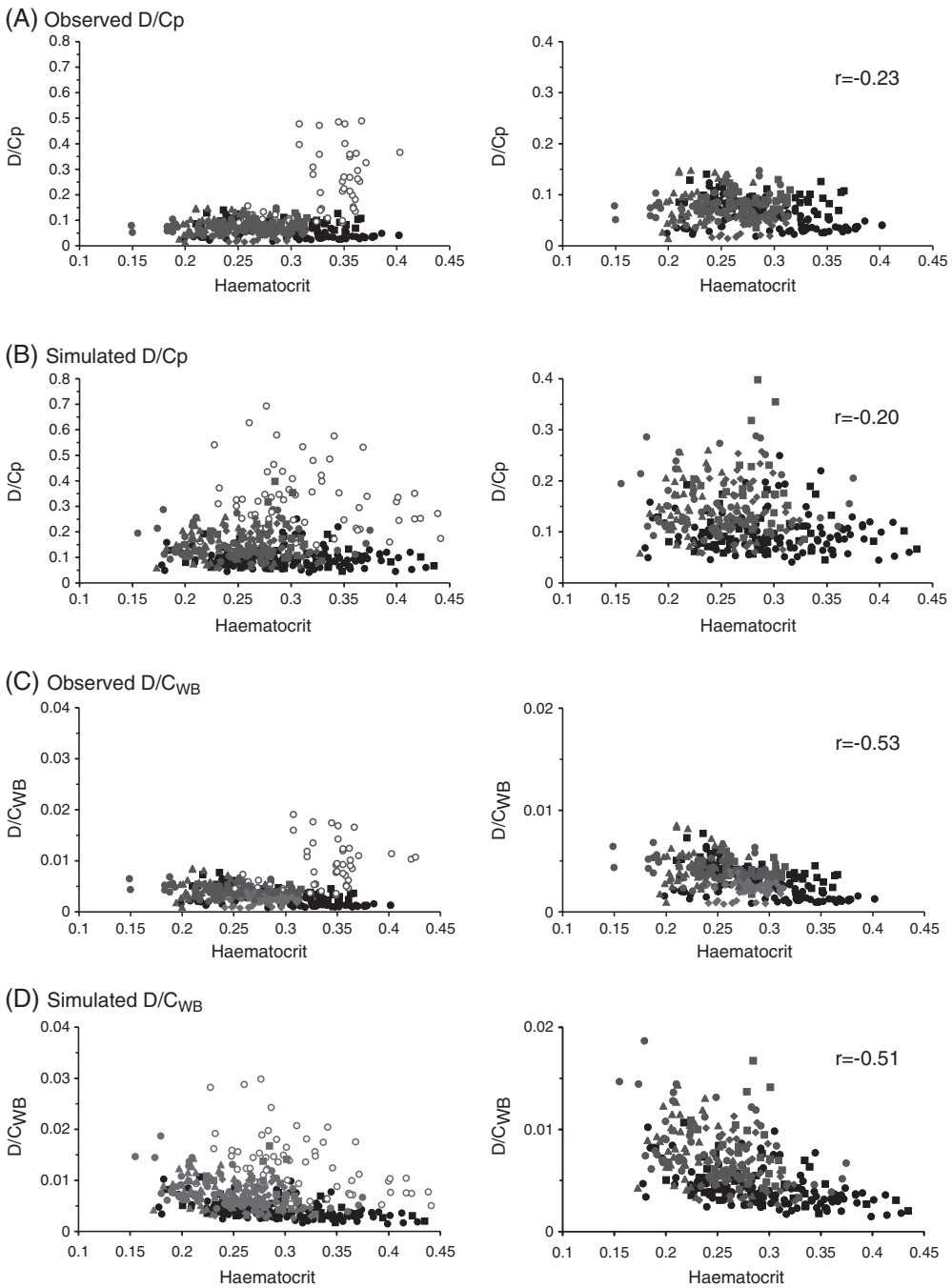

Figure 1. Comparison of the observed (A, C) and simulated (B, D) relationship between haematocrit and D/C of tacrolimus in plasma (D/Cp) (A, B) and whole blood (D/C_{WB}) (C, D). The observed data (A, C) were adopted from our previous report [8]. The right panels are enlargement without data of patient 4 (open circle). Black and gray symbols represent the data from male and female patients, respectively. r represents the correlation coefficient.

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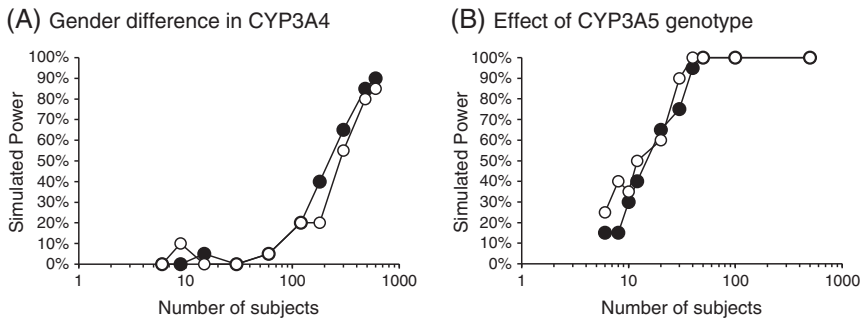

Figure 2. Influence of the number of subjects on the power of simulated studies of tacrolimus in blood and plasma to detect (A) sex difference in CYP3A4 content and (B) effect of CYP3A5 genotype using Japanese liver transplant patients. Open and closed circle represent the value for studies using whole blood and plasma, respectively

between D/C_{WB} and D/C_p was observed either in the detection of sex or CYP3A5 genotype effects.

Discussion

The primary objective of this study was to recover the impact of haematocrit on the clearance of tacrolimus. Therefore, the study investigated whether a population-based pharmacokinetic simulator (Simcyp®) can reproduce the observed relationship between haematocrit and D/C value of tacrolimus in liver transplant patients. This required some modifications to the simulation procedure such that typical 'interindividual' variations created by the simulator were representative of the 'intraindividual' variability (see Methods). It should be noted that D/C is not a pure reflection of differences in clearance. The trough levels are in particular influenced by drug distribution. Hence, the link between D/C and clearance would be strongest when the variation in the volume of distribution was minimal.

The impact of the haematocrit on clearance according to the assumption of the well-stirred liver model is well known (see Appendix). These have been the basis of the calculations for population pharmacokinetics involving the IVIVE and variability in haematocrit. Therefore, it should be noted that the simulator relies on first principles governing the pharmacokinetics (using IVIVE linked with PBPK). Using these basic assumptions, the predicted relationship (i.e. the magnitude and pattern) were similar to those observed as shown by similar coefficients of correlation for simulated results compared with those from observed data (Figure 1). Nonetheless, the model overestimated the absolute D/C values to some extent (although still within two-fold of observed values).

It was shown that a population-based pharmacokinetic simulation approach is useful in estimating not only the population mean of pharmacokinetic characteristics but also their interindividual variation [26]. The present study first successfully reproduced the observed intraindividual variation of clearance in liver transplant patients. Hence, the mechanistic approach based on IVIVE is considered useful for estimating the influence of not only the interindividual but also intraindividual variations of covariates. Whilst a data set from seven individuals might be considered insufficient to evaluate the interindividual variations in the first instance, the total number of samples was 403 (28–106 for each subject) and the variation in haematocrit was considerably large, due to the time-dependent change after surgery [10]. Hence, the relatively large variations in haematocrit enabled us to identify the relationship between the haematocrit and D/C value, which is expected in a larger population (i.e. large intraindividual variability acted as a surrogate for interindividual variation).

In our previous study, one patient (patient 4) was excluded from the analysis due to an 'extraordinarily high D/C value'. Although that individual was not genotyped, assuming the EM phenotype for CYP3A5 as reflection of the genotype, the clearance was simulated and some consistency was found between the observed and simulated data. This could provide, at least in part, an

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Table 2. Summary of the previous POPPK researches investigating the influence of CYP3A5 genotype and sex on the pharmacokinetics of tacrolimus in liver transplant patients

	Study country/ population	Number of samples	Number of subjects	Detection		Ref
				CYP3A5	Sex	
1	Asian/paediatrics	771	16	NA	NS	1
2	Japan	824	35	NA	NS	2
3	Australia	1742	68	NA	NS	3
4	France	728	37	NA	NS	4
5	Asia	213/157[a]	31/29[a]	NA	NS	5
6	Japan	3054	100	$p < 0.005$	NS	6
7	China	703	72	$p < 0.05$	$p < 0.05$[b]	7
8	Belgium	190	19	$p < 0.001$	NS	8
9	France	289	50	$p < 0.01$	NS	9

NA, not assessed; NS, no statistically significant influence was detected.
[a]Whole blood/plasma.
[b]Statistically significant only in the first step. Not statistically included as a covariate for further combined model.

explanation for the patient's high D/C value. Thus, based on the present simulation, it was not rational to have regarded the patient as an outlier. Many recent POPPK studies in fact have confirmed the impact of CYP3A5 as a covariate for kinetics of tacrolimus [6–9,15].

It was also reported that a population-based pharmacokinetic simulation approach is useful in estimating the statistical power of clinical studies to detect the influence of covariates [13,15]. The present results suggest that detecting the influence of CYP3A5 genotype is feasible, while the sex effect was considered to be less detectable unless very high numbers were used. This was regardless of the fact that the abundance of CYP3A4 was set to be 45% higher in female. These results are consistent with the results of previous POPPK studies carried out with liver transplant patients (Table 2). The effect of sex was not incorporated into the final POPPK model in any of seven studies, while the influence of CYP3A5 genotype was judged to be significant in all of studies with CYP3A5 genotyping. These results are valuable in the design of studies regarding the enrollment of adequate numbers of subjects into clinical studies. Liver transplant patients have large variations in haematocrit, which increases the variability of tacrolimus D/C values and may lead to a reduction in the power to detect the influence of covariates. Finally, the analysis of plasma drug concentration, instead of blood drug concentration, did not improve the statistical power in detecting the haematocrit effect noticeably (Figure 2). Therefore, to increase the power of detection, it may be important to monitor the haematocrit for each sample.

In conclusion, population based pharmacokinetics with IVIVE is considered to be a useful approach for assessment of the influence of covariates prior to conduct and design of POPPK. The statistical power estimated by a mechanistic approach was consistent with the results of previous tacrolimus POPPK research with liver transplant patients, who have large intraindividual variations of haematocrit and should be carefully enrolled to pharmacokinetic studies involving tacrolimus. Hence, using population-based mechanistic IVIVE approaches can inform the design of POPPK studies to a great extent.

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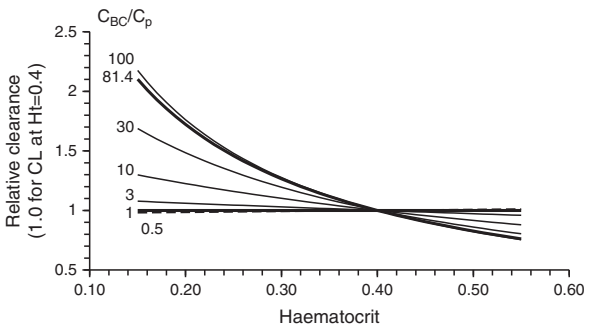
Appendix

Simulation of the impact of haematocrit (Ht) on the relative systemic blood clearance of tacrolimus (CL) (a well-stirred liver model is assumed), along with the influence of blood cell-to-plasma concentration ratio (C_{BC}/C_p). Note that C_{BC}/C_p value of tacrolimus is reported to be 81.4 [10]. Other pharmacokinetic properties such as plasma unbound fraction (f_u) were fixed



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