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ARTICLE

Population pharmacokinetics of everolimus in adult liver transplant patients: Comparison to tacrolimus disposition and extrapolation to pediatrics

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Abstract

Everolimus has recently been used to prevent graft rejection in liver transplantation and reduces the incidence of kidney dysfunction caused by calcineurin inhibitors. In this study, a population pharmacokinetic analysis was conducted to improve the individualization of everolimus therapy. Japanese post-liver transplant patients whose blood everolimus concentrations were measured between March 2018 and December 2020 were included in this study. A nonlinear mixed-effect modeling program was used to explore covariates that affect everolimus pharmacokinetics. Individual everolimus pharmacokinetic parameters estimated by the post-hoc Bayesian analysis using the final model were compared with the tacrolimus dose per trough concentration (D/C) ratio in each patient. The final model was extrapolated to pediatric liver transplant patients for external evaluation. A total of 937 concentrations from 87 adult patients were used in the model-building process. Everolimus clearance was significantly affected by the estimated glomerular filtration rate, concomitant use of fluconazole, sex, as well as total daily dose of everolimus (TDM effect). The estimated individual apparent clearance of everolimus by the post-hoc Bayesian analysis was moderately correlated with the D/C ratio of tacrolimus in each patient ($R^2 = 0.330$, $p < 0.0001$). The estimation accuracy in pediatric patients was considerably high, except for one infant out of 13 patients. In conclusion, population pharmacokinetic analysis clarified several significant covariates for everolimus pharmacokinetics in liver transplant patients. Everolimus pharmacokinetics moderately correlated with tacrolimus pharmacokinetics and could be extrapolated from adult to pediatric patients by body size correction, except for infants.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Therapeutic drug monitoring (TDM) of everolimus is needed because of its large inter- and intra-individual variability and narrow therapeutic window. There are several reports on population pharmacokinetic analysis of everolimus in renal transplantation. Additionally, a good correlation was found between the dose-adjusted area under the curve of tacrolimus and everolimus in Japanese renal transplant patients. However, there are few reports on everolimus pharmacokinetics in liver transplant patients.

WHAT QUESTION DID THIS STUDY ADDRESS?

Our study aimed to construct a population pharmacokinetic model of everolimus in adult liver transplant patients, investigate covariate factors influencing its pharmacokinetics, and evaluate the correlation between the individual apparent clearance of everolimus and the dose per trough concentration (D/C) ratios of tacrolimus in the same patients. We also examined the possibility of extrapolation of adult pharmacokinetics to pediatric patients using body size correction.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Estimated glomerular filtration rate, concomitant use of fluconazole, and sex are significant covariates for everolimus apparent clearance in adult liver transplant patients. Total daily dose of everolimus is also extracted as a significant covariate, indicating a TDM effect. The estimated everolimus individual apparent clearance is moderately related to the D/C ratio of tacrolimus in each patient. The constructed model could be extrapolated from adult to pediatric patients, except in infancy.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This study contributes to a better understanding of everolimus pharmacokinetics in adult and pediatric liver transplant patients. Target blood concentration of everolimus could be achieved at 0.5 mg twice daily in adult liver transplant patients.

INTRODUCTION

Everolimus is a rapamycin derivative that inhibits the mammalian target of rapamycin,[1] and it has been used as an immunosuppressant for kidney and heart transplantation for a long time. Liver transplant recipients are at a high risk of developing chronic renal failure,[2] and one of the risk factors for post-transplant renal dysfunction is exposure to calcineurin inhibitors such as tacrolimus. Recently, it has been reported that the introduction of everolimus with reduced exposure to tacrolimus therapy at a month post-liver transplantation has a clinically relevant renal benefit, comparable efficacy, and no safety concerns.[3–6] In February 2018, everolimus was approved for the prevention of graft rejection in liver transplantation in Japan. In liver transplant patients, everolimus is recommended to be started after the fourth post-operative week because it causes delayed wound healing.

Therapeutic drug monitoring (TDM) for the everolimus treatment is needed because of its large inter- and intra-individual variabilities and narrow therapeutic window.[7] There are several reports on the population pharmacokinetic (PPK) analysis of everolimus in renal transplantation,[8,9] but there are no reports on its PPK in liver transplantation. Hepatic clearance may fluctuate after liver transplantation, and we experienced many cases in which blood concentrations of everolimus exceeded the therapeutic range of 3–8 ng/ml when starting at the labeled dose for liver transplant patients. Therefore, PPK analysis of everolimus for liver transplant patients is important for accurate dosing design. On the one hand, a good correlation has been found between the dose-adjusted area under the curve (AUC/D) of tacrolimus and everolimus in Japanese renal transplant patients.[10] Therefore, blood concentration monitoring data of tacrolimus may help determine the everolimus dosage if this relationship is observed in liver transplant patients, although no previous reports on this relationship have been reported in liver transplant patients. Everolimus is sometimes used in pediatric liver transplantation;

however, there are few reports on the pharmacokinetics of everolimus in the pediatric population.

In this study we performed a PPK analysis of everolimus in adult liver transplant patients to investigate the covariate factors influencing its pharmacokinetics. Next, we evaluated the correlation between the estimated individual apparent clearance (CL/F) of everolimus and the dose per trough concentration (D/C) ratios of tacrolimus in the same patients. Additionally, we examined the possibility of extrapolation of adult pharmacokinetics to pediatric patients using body size correction.

METHODS

Patients and data collection

Japanese post-liver transplant patients who were started on everolimus and whose blood concentrations were measured at Kyoto University Hospital between March 2018 and December 2020 were included in this study. Patients who underwent plasma exchange or were followed up at other hospitals were excluded. Patients who used tofisopam concomitantly was excluded. This was because everolimus trough concentration was clearly elevated when tofisopam was used concomitantly, but only one patient used tofisopam and it was not appropriate to estimate a reliable interaction effect. For each patient the following data were retrospectively collected from electronic medical records: blood concentrations and dose of everolimus and tacrolimus, time of dosing and sampling, age, height, body weight, sex, post-operative day, clinical laboratory data (e.g., hematocrit, aspartate aminotransferase [AST], alanine aminotransferase [ALT], albumin, and estimated glomerular filtration rate [eGFR]), and concomitant drugs (e.g., cyclosporine, fluconazole, nifedipine, and prednisolone). These concomitant drugs, which interact with everolimus or CYP3A, were selected based on drug history during the observation period. The collected data were divided into adults (>16 years) and children (≤16 years) groups. The eGFR of each Japanese adult was calculated using the following equation[11]:

$$\mathrm{eGFR} = 194 \times \mathrm{Scr}^{-1.094} \times \mathrm{Age}^{-0.287} \times 0.739 \ (\text{if female})$$

where Scr is the serum creatinine concentration. The eGFR for each Japanese child was calculated using the following equations, as reported previously[12,13]:

$$\mathrm{eGFR} = \left(110.2 \times \left(\frac{\mathrm{refCr}}{\mathrm{Scr}}\right) + 2.93\right) \times \mathrm{R} \ (\text{if Age} \leq 2 \text{ years})$$

$$R = 0.107 \times \ln(\mathrm{Age(months)}) + 0.656$$

where refCr is the value of the reference Scr concentration and HT is height.

$$\begin{cases} \mathrm{refCr} = -1.259\mathrm{HT}^5 + 7.815\mathrm{HT}^4 - 18.57\mathrm{HT}^3 + 21.39\mathrm{HT}^2 - 11.71\mathrm{HT} + 2.628 \ (\text{male}) \\ \mathrm{refCr} = -4.536\mathrm{HT}^5 + 27.16\mathrm{HT}^4 - 63.47\mathrm{HT}^3 + 72.43\mathrm{HT}^2 - 40.06\mathrm{HT} + 8.778 \ (\text{female}) \end{cases}$$

The initial dose of everolimus was determined at the discretion of the transplant team, and 0.5 or 1.0 mg was usually administered twice a day. Since the exact time of oral administration and blood collection could not be extracted from the electronic medical record, the timing of everolimus administration was assumed to be basically at 9:00 a.m./9:00 p.m., and for blood collection days, it was assumed that everolimus was taken after blood collection if the blood collection time was after 9:00 a.m. In addition, blood collection time was assumed to be 7:00 a.m. for inpatients and the measured time of blood concentration for outpatients. More than one sampling points at a single dose interval were not obtained in each patient. The concentration of everolimus in the whole blood was measured using an automated electrochemiluminescence immunoassay (Cobas; Roche Diagnostics K.K., Tokyo, Japan). The lower and upper limits of quantification were 0.5 and 30 ng/ml, respectively. The everolimus dose was adjusted to maintain trough concentrations in the range 3–8 ng/ml. For immunosuppressive therapy, a calcineurin inhibitor (tacrolimus or cyclosporine) and mycophenolate mofetil were used for most patients. Other immunosuppressive drugs such as prednisolone and azathioprine were also used in some patients.

This study was carried out in accordance with the Declaration of Helsinki and its amendments, and was approved by the Ethics Committee of Kyoto University Graduate School, Faculty of Medicine, and Kyoto University Hospital (No. R0545-2).

PPK modeling

PPK analysis was conducted using the nonlinear mixed-effects modeling (NONMEM) program version 7.5.0 (ICON, Ellicott City, MD) with the first-order conditional estimation method with interaction. Perl-speaks-NONMEM version 5.0.0 and R 4.0.3 (R-project.org) were used to evaluate the goodness of fit and visualize the output. The structural model used was a one-compartment model, and the proportional error model was used to

determine the inter-individual variability (IIV) of the pharmacokinetic parameters. The residual variability in blood concentrations was compared between the additive, proportional, and mixed (additive and proportional) error models. The residual error model selection was based on the Bayesian information criteria (BIC). Because of insufficient data in the absorption phase, the absorption rate constant (ka) was fixed at 6.07 h^{-1} based on the value in the literature.[8] To account for the effect of body size, allometric scaling was used for CL/F and apparent volume of distribution (Vd/F). The allometric coefficients for the effect of body weight on CL/F and Vd/F were fixed at 0.75 and 1, respectively, based on the previous literature.[14]

To assess the influence of continuous covariates, such as age, post-operative day, total daily dose (TDD; mg/day) of everolimus, and clinical laboratory data on CL/F, and hematocrit and albumin on Vd/F, covariate analysis was conducted using the following equation:

$$\theta_{tv} = \theta_p \times \left(COV/COV_{med}\right)^{\theta_{cov}}$$

where θ_{tv} is the typical value of the pharmacokinetic parameters (such as CL/F and Vd/F), θ_p is the mean parameter to be estimated, and θ_{cov} is the factor contributed by the covariate. COV indicates the actual value for each patient, and COV_{med} is the median value of the baseline clinical data. To assess the influence of categorical covariates such as sex and presence of a concomitant drug on CL/F, and sex on Vd/F, covariate analysis was conducted using the following equation:

$$\theta_{tv} = \theta_p \times \theta_{cov}{}^{COV}$$

where COV is 1 for females or 0 for males when evaluating the effect of sex, and COV is 1 when the drug of interest was used, or 0 when evaluating the effect of concomitant drug use.

The influence of each covariate on CL/F or Vd/F was evaluated based on the difference in the objective function value (OBJ) between the previous model and the model that included the covariate. The OBJ changes were considered significant at a minimum value of 3.84 (χ^2 test; $p < 0.05$) and 6.63 (χ^2 test; $p < 0.01$) per additional parameter in the stepwise forward inclusion and stepwise backward elimination methods, respectively. Additionally, the relative standard errors for parameter estimates and shrinkage of IIV were calculated and considered in the model selection. The individual predicted CL/F and concentration were obtained using the empirical Bayesian estimation in the first-order conditional estimation method.

Model evaluation

The final model was evaluated using a goodness-of-fit plot and prediction-corrected visual predictive checks (pcVPC).[15] The goodness-of-fit plots were as follows: observed concentration (OBS) versus population predicted value (PRED) or individual predicted value (IPRED), conditional weighted residuals (CWRES) versus PRED, time, or time after last dose to identify any bias corresponding to model misspecification. To assess the predictability of the final model, the pcVPC was performed using a simulation of 1000 datasets from the final model. The 2.5th, 50th, and 97.5th percentile curves of the observed data were overlaid on the 95% confidence interval of the 2.5th, 50th, and 97.5th simulated percentiles and visually evaluated. Additionally, the estimated final model parameters were assessed by bootstrapping. Bootstrapping was conducted using 500 datasets with 87 subjects randomly sampled (with replacement) from the 87 subjects in the original dataset. The model parameters were estimated for each bootstrap replicate using the NONMEM.

Monte Carlo simulation

The trough concentrations of everolimus based on the final model were calculated using Monte Carlo simulation to determine the effects of body weight, eGFR, sex, and concomitant use of fluconazole. Five thousand pharmacokinetic profiles were simulated for patients with various body weights (30, 60, and 90 kg), eGFR (15, 30, 60, and 90 ml/min/1.73 m^2), concomitant use of fluconazole, and each sex. The simulated dose of everolimus was set at 0.5 mg twice daily, and body weight and eGFR were fixed to the median value of the population (57 kg and 55.2 ml/min/1.73 m^2) for the effect of eGFR and body weight, respectively.

Correlation between estimated individual CL/F of everolimus and D/C ratio of tacrolimus in the same patient

The individual pharmacokinetic parameters were estimated by the post-hoc Bayesian method using the final model. Patients who received concomitant tacrolimus at the start of everolimus administration were selected, and the D/C ratio of tacrolimus was calculated using the trough concentration and dose of tacrolimus observed just before the start of everolimus administration. The regression line between the individual everolimus CL/F and the tacrolimus D/C ratio was calculated, and the variation

was expressed as a coefficient of determination (R^2). In addition, a covariate analysis was performed to determine if the prior tacrolimus D/C ratio was a predictor of everolimus CL/F in liver transplant patients who used tacrolimus before the start of everolimus.

Extrapolation to pediatric patients

The predicted everolimus concentrations in all children were calculated using the final model and were compared with their observed concentrations. To assess the bias and precision, OBS versus PRED and OBS minus PRED versus age plots were analyzed, and the prediction error (PE) and mean prediction error (MPE) were calculated using the first sampling concentration for each patient as described below[16]:

$$PE_i = OBS_i - PRED_i$$

$$MPE = \frac{1}{N}\sum_{i=1}^{N} PE_i$$

where PE_i is the PE for the ith patient, and OBS_i and $PRED_i$ are the OBS and population predicted concentrations of everolimus corresponding to the observed data in the ith patient, respectively. N is the number of patients.

RESULTS

Patient characteristics

The patient characteristics before everolimus therapy are summarized in Table 1. A total of 100 patients were included in this study. The patients were divided into two groups: adults (>16 years) and children (≤16 years). A total of 937 concentrations from 87 adult patients were used in the model-building process. A total of 373 concentrations from 13 pediatric patients were used for validation. The median ages of the adult and pediatric patients were 60 (20–77) and 6 (0.75–16) years, respectively. The numbers of patients who were administered cyclosporine, fluconazole, prednisolone, and nifedipine during the observation period in the adult group were 5, 21, 40, and 6 subjects, respectively.

TABLE 1 Patient characteristics at the first sampling

Characteristic	Adult	Child
Male/female, *n*	41/46	6/7
Age, years	60 (20–77)	6 (0.75–16)
Body weight, kg	57.0 (34.5–91.0)	16.9 (7.3–58.0)
Post-operative day, days	1865 (29–8092)	608 (19–5029)
Hematocrit, %	33.0 (18.8–51.4)	33.3 (22.5–47.2)
AST, U/L	26 (10–177)	45 (19–118)
ALT, U/L	23 (4–380)	66 (17–299)
Albumin, g/dl	3.8 (1.6–5.1)	3.8 (2.5–4.4)
eGFR, ml/min/1.73 m^2	55.2 (11.4–223.7)	129 (85.4–216)
Everolimus TDD, mg/day	1.0 (0.25–4.0)	0.5 (0.25–1.0)
Concomitant drug, *n* (%)		
Cyclosporine	5 (5.75)	1 (7.69)
Fluconazole	21 (24.1)	2 (15.4)
Prednisolone	40 (46.0)	11 (84.6)
Nifedipine	6 (6.90)	1 (7.69)

Note: Values are presented median (min−max) for continuous variables.
Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; TDD, total daily dose.

PPK modeling and model evaluation

A one-compartment model described the everolimus concentration data reasonably well. For IIV, an exponential error model for CL/F was included in the model. The shrinkage value of IIV for Vd/F was 49%, and this IIV was excluded because shrinkage >20%–30% would lack informativeness and may be misleading.[17] For residual variability, the BIC values of additive, proportional, and mixed (additive and proportional) error models were 1684, 1686, and 1619, respectively. The mixed (additive and proportional) error model was selected because the BIC value was minimal. The results of the covariate analyses are shown in Table S1. After the forward inclusion and backward elimination steps, everolimus CL/F was significantly affected by TDD, concomitant use of fluconazole, eGFR, and sex. The final models for CL/F and Vd/F were as follows.

$$CL/F\,(L/h) = 6.89 \times \left(\frac{BW}{57}\right)^{0.75} \times \left(\frac{TDD}{1.0}\right)^{0.310} \times 0.823^{FLCZ} \times \left(\frac{eGFR}{55.2}\right)^{0.258} \times 1.23^{Female}$$

$$Vd/F\,(L) = 312 \times \left(\frac{BW}{57}\right)$$

where BW is body weight. FLCZ is 1 when fluconazole is used concomitantly and 0 otherwise. Female is 1 for female patients and 0 for male patients. The final estimates of the PPK parameters for everolimus, including the relative standard errors and shrinkage values, are listed in Table 2. The inclusion of these covariates improved the model, as the

TABLE 2 Population pharmacokinetic parameters of everolimus

	Final model			Bootstrap results ($N = 500$)	
Parameter	**Estimates**	**RSE, %**		**Median**	**95% CI**
$CL/F = \theta_1 \times (BW/57)^{0.75} \times (TDD/1.0)^{\theta 2} \times \theta_3^{FLCZ} \times (eGFR/55.2)^{\theta 4} \times \theta_5^{Female}$					
θ_1 (L/h)	6.89	4.63		6.89	6.23–7.65
θ_2 (TDM effect)	0.310	15.6		0.316	0.235–0.432
θ_3	0.823	5.13		0.820	0.722–0.903
θ_4	0.258	24.7		0.248	0.144–0.404
θ_5	1.23	6.24		1.23	1.10–1.39
$Vd/F = \theta_6 \times (BW/57)$					
θ_6 (L)	312	9.65		313	258–388
ka (1/h)	6.07 (Fixed)			6.07 (Fixed)	
	Estimates	**RSE, %**	**Shrinkage**	**Median**	**95% CI**
Interindividual variability, CV%					
IIV for CL	27.1	17.4	4.67	26.5	21.8–31.8
Residual variability,					
Proportional error, CV%	18.9	15.6	4.06	18.6	15.2–21.4
Additive error, ng/ml	0.495	40.7	4.06	0.499	0.281–0.684

Abbreviations: BW, body weight; CI, confidence interval; CL/F, apparent clearance (L/h); CV, coefficient of variation; eGFR, estimated glomerular filtration rate; FLCZ, fluconazole; IIV, inter-individual variability; RSE, relative standard error; TDD, total daily dose of everolimus (mg/day); TDM, therapeutic drug monitoring; Vd/F, apparent volume of distribution (L). FLCZ is 1 when fluconazole is used concomitantly and 0 otherwise. Female is 1 for female patient and 0 for male patients.

ΔOBJ between the base and final models was approximately −328.

Of the 500 bootstrap runs performed for the model evaluation, the percentage of successful runs was 100%. The median values of the bootstrap replicates and final parameter estimates were similar (Table 2), indicating that the final parameters were properly estimated. The goodness-of-fit plots of the final model are shown in Figure 1. The plot of OBS versus PRED or IPRED approached unity. Moreover, CWRES was evenly distributed around zero against PRED, time, and time after the last dose. The pcVPC plot for everolimus concentration versus time after the last dosing showed that the model described the central tendency and variability of the data (Figure 2). The number of blood concentrations for each period of post-dose was as follows: 0–9.9 h ($n = 0$), 10–10.9 h ($n = 260$), 11–20.9 h ($n = 628$), 21–25.9 h ($n = 39$), and ≥26 h ($n = 10$).

Monte Carlo simulation

Figure 3 shows the trough concentrations simulated based on the final model for patients receiving everolimus 0.5 mg twice daily with various body weights, eGFR for each sex, and concomitant use of fluconazole. The median trough concentrations were almost within the target concentration of 3–8 ng/ml. However, the median trough concentration was beyond the upper limit of the target range for male patients who had a light body weight. In contrast, the median trough concentration was lower than the target range for female patients who were not being administered fluconazole and who had heavy body weight.

Relationship between estimated individual CL/F of everolimus and D/C ratio of tacrolimus

Figure 4 shows the relationship between the individual everolimus CL/F estimated using the post-hoc Bayesian method in the final model and the D/C ratio of tacrolimus just before the start of everolimus administration in the same patient ($n = 80$). After the linear regression analysis, the R^2 value was 0.330 ($p < 0.0001$).

In addition to the original covariates in Table S1, the D/C ratio of tacrolimus was extracted as a significant covariate for CL/F, and the final models were as follows:

$$CL/F\,(L/h) = 7.04 \times \left(\frac{BW}{57}\right)^{0.75} \times \left(\frac{TDD}{1.0}\right)^{0.338} \times 0.803^{FLCZ} \times \left(\frac{eGFR}{55.2}\right)^{0.293} \times \left(\frac{D/C\ Tac}{0.44}\right)^{0.230} \times 1.23^{Female}$$

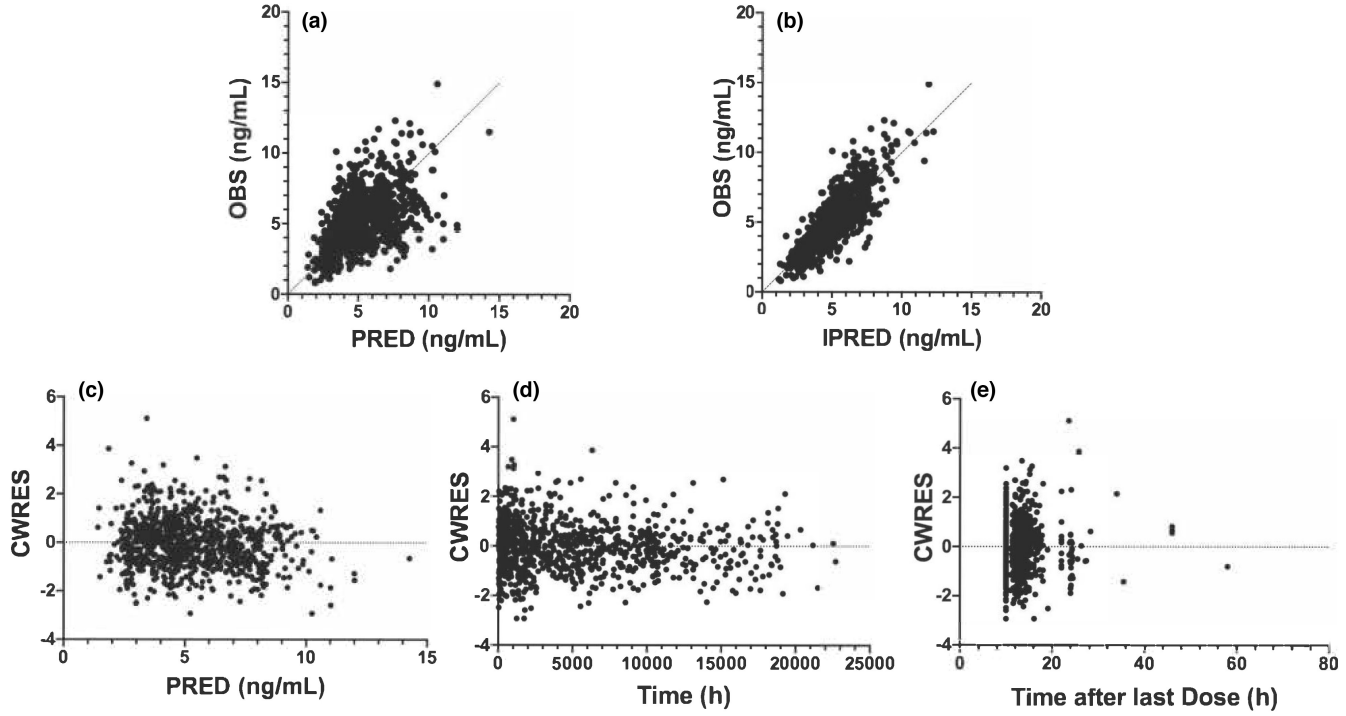

FIGURE 1 Goodness-of-fit plots for the final model. The observed (OBS) versus PRED (a) and IPRED (b); conditional weighted residuals (CWRES) versus PRED (c) and time (d), and time after last dosing (e). Each line in (a) and (b) represent a line of unity.

$$\mathrm{Vd/F\,(L)} = 323 \times \left(\frac{\mathrm{BW}}{57}\right)$$

when the D/C ratio of tacrolimus was included the model, the IIV for CL/F decreased to 23.4%. The proportional error and additive error for residual variability were estimated to be 18.2% and 0.510 ng/ml, respectively, which were almost the same values as in the original model.

Extrapolation to pediatric patients

Figure 5 shows OBS versus PRED and OBS minus PRED versus age plots estimated using the final model for the 13 pediatric patients. In one patient, a large difference between the PRED and OBS was observed, and the OBS in this outlier patient is shown as an open circle in Figure 5. The age and body weight of this patient were 9 months and 7.7 kg, respectively, when everolimus was initiated. In this patient, the values of OBS minus PRED were largely negatively biased below 2 years of age (Figure 5b). The MPE value for 13 pediatric patients was −1.96 (SD: 4.81) ng/ml. When the outlier patient's data were removed from the calculation, the MPE value for the 12 children was −0.706 (SD: 1.76) ng/ml.

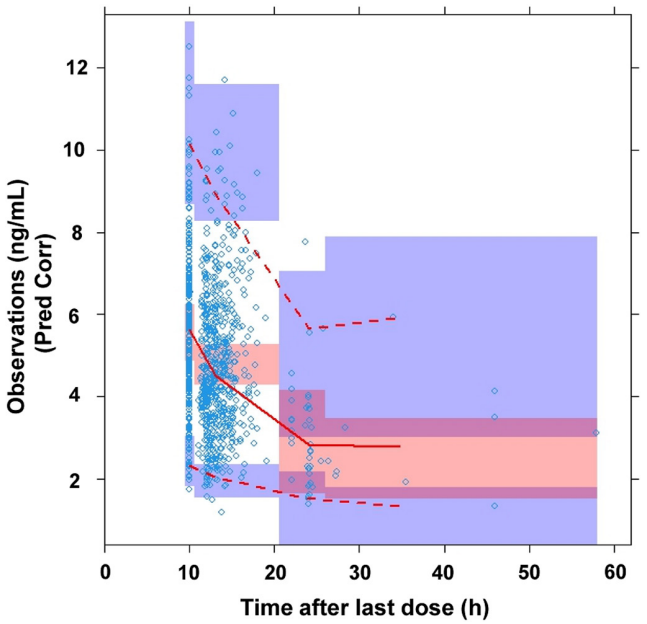

FIGURE 2 Prediction-corrected visual predictive checks (pcVPC) of everolimus observed data compared with 500-replication datasets obtained from the final model. Open circles represent the observed data. The solid and dotted lines denote the 50th and 2.5th or 97.5th percentiles of the observed data, respectively. The shaded areas denote the 95% confidence intervals of the 2.5th, 50th, and 97.5th percentiles of the simulated data.

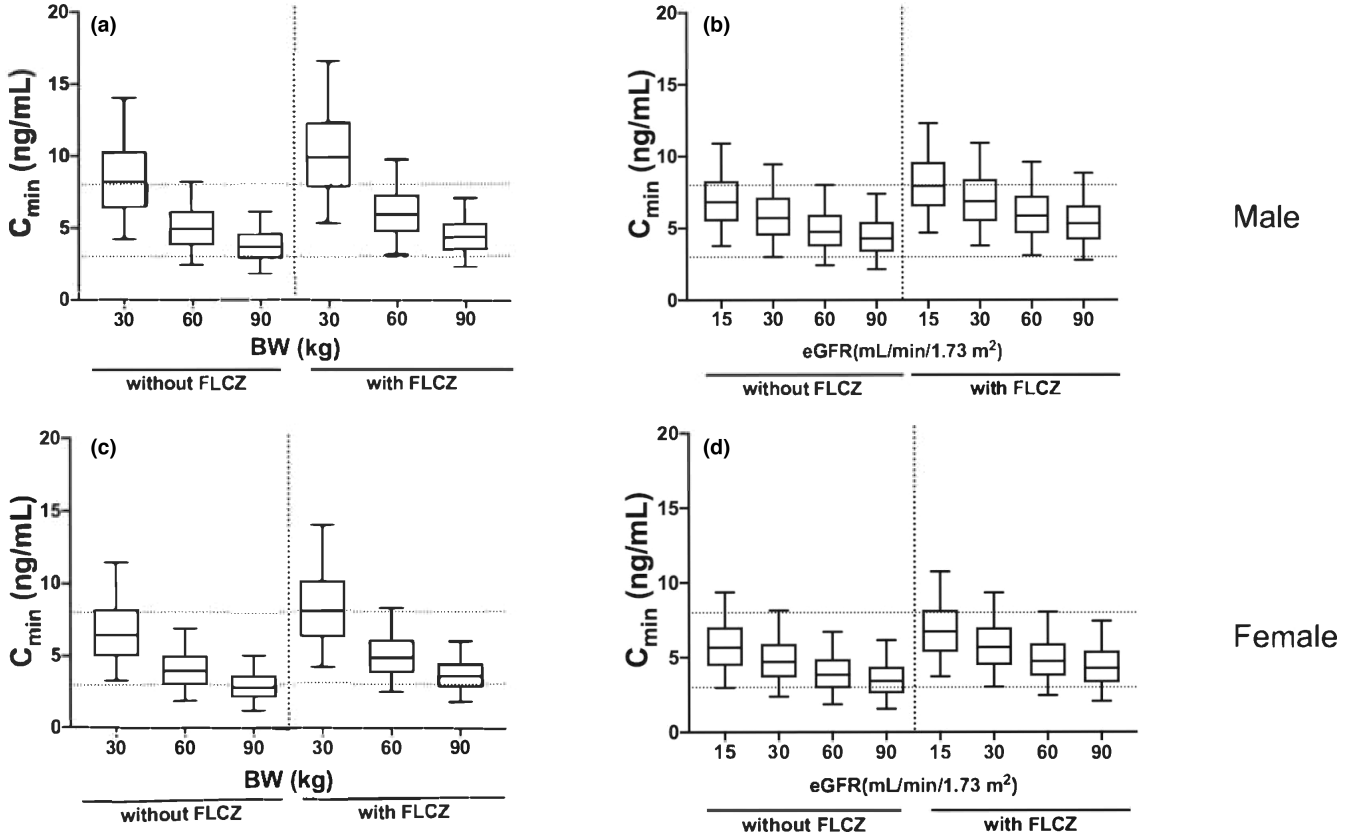

FIGURE 3 Simulation of trough concentration (C_{min}) of everolimus in the 5000-replication datasets in a typical patient receiving everolimus 0.5 mg twice daily classified by body weight (BW), estimated glomerular filtration rate (eGFR), sex, and concomitant use of fluconazole (FLCZ). Each box plot represents the interquartile range and 90% prediction interval of the predicted trough concentration of everolimus.

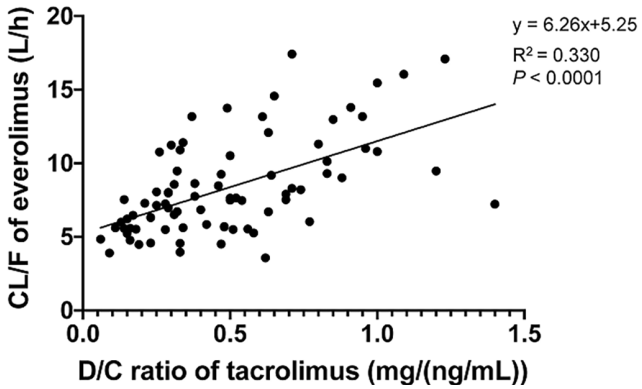

FIGURE 4 Correlation between the estimated individual apparent clearance (CL/F) of everolimus using the post-hoc Bayesian method using the final model and dose per trough concentration (D/C) ratio of tacrolimus in 80 patients.

DISCUSSION

To the best of our knowledge, this is the first PPK analysis of everolimus in liver transplant patients. The PPK model of everolimus in adult liver transplant patients was constructed using routinely obtained clinical data, and showed that eGFR, sex, concomitant use of fluconazole as well as TDD were significant covariates of everolimus pharmacokinetics. The effects of TDD and fluconazole on everolimus CL/F have been reported previously,[9,18] but this is the first PPK report showing that eGFR and sex were significant covariates of everolimus CL/F. According to previous PPK studies of everolimus in renal and heart transplant patients whose body weight range was 40–131 kg, the values of the steady-state volume of distribution were in the range 110–646 L.[8,9,19] In this study, patients' body weight range was 34.5–91 kg and Vd/F was estimated as 5.5 L/kg, similar to previous reports. However, the reported CL/F values were 13.5–17.9 L/h in renal or cardiac recipients if concomitant drug administration was not considered,[9,19] showing a slightly higher value than our result (6.89 L/h). We have previously reported that hepatic function in liver transplant patients would be slightly lower than that in renal transplant patients.[20] Therefore, the lower CL/F value may be due to differences in the target population.

Patients with a lower eGFR had a decreased CL/F of everolimus. Although everolimus and tacrolimus are

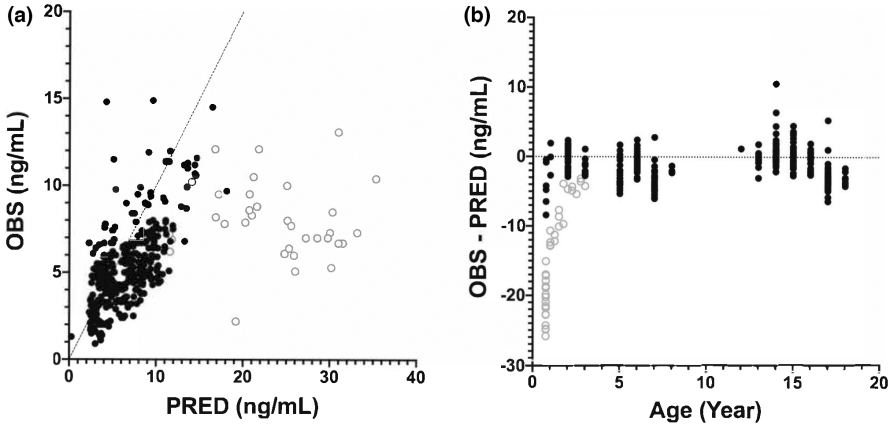

FIGURE 5 Goodness-of-fit plots for the pediatric patients using the final model. The observed (OBS) versus PRED (a) and OBS minus PRED versus age (b). Each closed circle denotes the observed concentration in 12 patients. Each open circle denotes the observed concentration in the outlier patient.

metabolized by CYP3A and minimum quantities are excreted in urine in an unchanged form,[7] some studies reported a significant negative correlation between serum creatinine and tacrolimus clearance.[21–23] Furthermore, a previous study has reported that uremic toxins in patients with renal failure decrease the activity of CYP3A4.[24] Based on this information, the finding that everolimus CL/F was decreased, when the renal function was impaired, seems reasonable. Since the dose adjustment for renal impairment is not indicated in the everolimus package insert, further studies are needed to confirm the effect of renal impairment on everolimus pharmacokinetics. In terms of sex differences, our study indicated that female patients had a 1.23 times higher CL/F than male patients. Some previous studies, which have been conducted on human liver microsomes prepared from men and women, have suggested that CYP3A4 content and activity are 1.2–2.0 times higher in women,[25–28] which is consistent with our results. Interestingly, TDD was extracted as a significant covariate for CL/F. In this study, everolimus dose was adjusted to attain a target concentration based on the measured everolimus concentration as routine TDM. Therefore, patients with high or low everolimus clearance would change to a higher or lower TDD, resulting in the TDM-induced correlation between everolimus clearance and TDD (namely, a TDM effect), as the previously published everolimus model developed with TDM data.[9] Importantly, it should be noted that this dose–clearance relationship is due to the strict TDM results and does not imply a nonlinearity of everolimus pharmacokinetics, as shown by Ahn et al.[29]

The present simulation studies of everolimus using the final model indicated that most of the median trough concentrations in several cases were within the therapeutic range when the dose was 0.5 mg twice daily. Although the dosage written in the package insert of everolimus is 1.0 mg twice daily, the present study indicated that target blood concentration could be achieved at 0.5 mg twice daily in adult liver transplant patients. Further investigations are required to determine the appropriate dosage in adult liver transplant patients because this was a retrospective, single-center study. The interaction between cyclosporine and everolimus CL/F is well known[19]; however, we could not confirm this in this study because of the small number of patients being administered cyclosporine.

A moderate correlation between the individual everolimus CL/F and the tacrolimus D/C ratio was observed, as shown in Figure 4. A previous study reported that a correlation between the AUC/D of tacrolimus and everolimus was found for patients with the *CYP3A5*1* allele ($R^2 = 0.587$) or *CYP3A5*3/*3* ($R^2 = 0.396$) 1 year after renal transplantation.[10] When we extracted the plots shown in this paper using WebPlotDigitizer ver 4.4 (https://automeris.io/WebPlotDigitizer), we obtained an R^2 value of 0.285 using all the data together, which was similar to our results. The mean tacrolimus D/C ratio (mg/[ng/ml]) just before (mean of 2 days before) and after the start of everolimus of 80 patients in Figure 4 was 0.49 (SD: 0.30) and 0.51 (SD: 0.32), respectively, showing no statistically significant differences ($p < 0.366$ by the paired *t*-test). We also performed the covariate analysis for patients who used tacrolimus before starting everolimus, and the tacrolimus D/C ratio was extracted as a significant covariate for the everolimus CL/F. Therefore, if a patient used tacrolimus before everolimus initiation, tacrolimus D/C ratios as prior information would be helpful in the initial dosing design of everolimus.

The estimation accuracy when the final model was used in children was considerably high, except in one infant (Figure 5). In this patient, the values of OBS minus PRED showed a largely negative bias below 2 years of age. The required dose to maintain the blood concentration within the therapeutic range early after everolimus initiation was as large as 2.0 mg/day before 1 year of age, but decreased to 1.5 mg/day and further decreased to 0.75 mg/day after 2 years of age (Figure S1), showing that the CL/F per body weight of this patient was quite high before 1 year of age and gradually decreased thereafter.

The precise reason why this patient showed such a high CL/F was unknown, but we believe that the high CL/F was caused by the low F in the infant transplant patient, although a relatively large grafted liver in small children might cause a larger CL, as mentioned in a study on tacrolimus.[30] Therefore, the constructed model could be extrapolated from adult to pediatric patients by body size correction; however, it should be noted that the pharmacokinetics of everolimus would differ in infants, which may lead to misprediction.

This study has several limitations. First, almost all the observed data were trough concentrations. Rich sampling data are required to explore covariates on the Vd/F or to describe the precise absorption profile in future studies. Second, this study had a limited sample size, especially in children, which makes it difficult to explore the effects of age-dependent changes, such as maturation or graft-to-recipient weight ratio, on CL/F. Therefore, further studies are needed to explore the precise pharmacokinetics in pediatric liver transplant patients. Third, information on the *CYP3A5* genotype could not be collected because this was a retrospective study. Everolimus is partly metabolized by CYP3A5,[31] therefore the *CYP3A5* genotype may affect everolimus pharmacokinetics. Fourth, the meal timing and content could not be controlled because both inpatients and outpatients were included in this study. Previous literature reported that a high-fat meal reduced the peak blood concentration and AUC of everolimus by 60% and 16%, respectively.[32] Therefore, further studies are needed to evaluate the effects of food on everolimus pharmacokinetics.

In conclusion, a PPK analysis of everolimus using routinely monitored data clarified the significant effects of eGFR, sex, and concomitant use of fluconazole in adult liver transplant patients. In addition, the inclusion of the TDM effect (TTD of everolimus) significantly improved the model fitting. The individual CL/F of everolimus was moderately correlated with the tacrolimus D/C ratio. Therefore, the baseline D/C ratio of tacrolimus may be helpful in determining the dosage of everolimus. The PPK model could be extrapolated to pediatric patients; however, further investigations are needed for infant liver transplant patients.

AUTHOR CONTRIBUTIONS

K.I., I.Y., A.Y., T.T., and K.M. wrote the manuscript. K.I. and I.Y. designed the research. K.I., I.Y., S.N., M.S., M.H., A.Y., S.I., T.N., D.H., T.I., K.H., E.H., T.T., and K.M. performed the research. K.I. analyzed the data.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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