Individualized dosing of calcineurin inhibitors in renal transplantation

Press, R.R.

Citation

Press, R. R. (2011, April 13). *Individualized dosing of calcineurin inhibitors in renal transplantation*. Retrieved from https://hdl.handle.net/1887/16715

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/16715

Note: To cite this publication please use the final published version (if applicable).

3

Rogier R Press, Bart A Ploeger, Jan den Hartigh, Tahar van der Straaten, Hans van Pelt, Meindert Danhof, Johan W de Fijter and Henk-Jan Guchelaar

Ther Drug Monitoring 2009; 31(2): 187-97

Explaining Variability in Tacrolimus Pharmacokinetics to Optimize Early Exposure in Adult Kidney Transplant Recipients

ABSTRACT

To prevent acute rejection episodes it is important to reach adequate tacrolimus exposure early after kidney transplantation. With a better understanding of the high variability in the pharmacokinetics of tacrolimus the starting dose can be individualized, resulting in a reduction in dose adjustments to obtain the target exposure. A population pharmacokinetic analysis was performed to estimate the effects of demographic factors, hematocrit, serum albumin concentration, prednisolone dose, tacrolimus dose interval, polymorphisms in genes coding for ABCB1, CYP3A5, CYP3A4 and the pregnane X receptor on tacrolimus pharmacokinetics. Pharmacokinetic data were prospectively obtained in 31 *de novo* kidney transplant patients randomized to receive tacrolimus once or twice daily and subsequently, the data were analyzed by means of Non-Linear-Mixed-Effects-Modelling. Tacrolimus clearance was 1.5 fold higher for patients with the CYP3A5*1/*3 genotype compare to the CYP3A5*3/*3 genotype (5.5±0.5L/h *versus* 3.7±0.3 L/h respectively). This factor explained 30% of the inter-individual variability in apparent clearance (exposure). Also, a relationship between the pregnane X receptor A+7635G genotype and tacrolimus clearance was identified with a clearance of 3.9±0.3 L/h in the A-allele carriers *versus* 5.4±0.6L/h in the GG genotype. Finally, a concomitant prednisolone dose of more than 10 mg/day increased the tacrolimus apparent clearance by 15%. In contrast body weight was not related to tacrolimus clearance in this population. As patients are typically dosed per kg body weight this might result in under- and overexposure in patients, with a low and high body weight respectively. This integrated analysis shows that adult renal transplant recipients with the CYP3A5*1/*3 genotype require a 1.5 times higher fixed starting dose compared to CYP3A5*3/*3 in order to reach the predefined target exposure early after transplantation.

Introduction

The calcineurin inhibitor (CNI) tacrolimus (TRL) is an important immunosuppressive drug commonly used in the early phases after solid organ transplantation. The current trend is to minimize exposure to CNIs, but at the same time to maintain the current low acute rejection rates [1]. This indicates that adequate early TRL exposure may become more important to prevent acute rejection episodes. Next to acute rejection, prevention of acute CNI-induced nephrotoxicity is another key objective, since both conditions were found to constitute the major risk factors for the occurrence of chronic allograft nephropathy in protocol biopsies obtained two years after renal transplantation [2].

CNIs are critical dose drugs with a large inter- and intra-individual variability in TRL pharmacokinetics (PK) and therewith systemic exposure [3,4]. In order to reach the target exposure of TRL as early as possible after transplantation it is important to have a clear understanding of the key factors (covariates) that explain variability in TRL exposure. Previous studies have identified a variety of demographic [3] and clinical factors (albumin [5,6], hematocrit [7,8]), co-medication (prednisolone [9-11], fluconazole [12]) as well as polymorphisms in genes encoding for CYP3A5 [13-17], CYP3A4 [18,19], P-glycoprotein (ABCB1/MDR1) [13,20] and the pregnane X receptor (PXR) [21-23] as relevant determinants of variability in TRL PK. The majority of these studies were limited by the fact that only a single factor was studied in relation to PK parameters such as area-under-the-concentration-over-time-curve (AUC), bioavailability or the clearance (CL/F) of TRL, while in most cases dose-adjusted trough levels were used. Recently, the importance of genotyping (CYP3A5) to reach TRL target exposure was emphasized by MacPhee *et al.* [24], but until now no population analysis has been performed in *de novo* kidney transplant recipients in which both genetic and non-genetic covariates were studied [25,26].

At present most transplant centers calculate the TRL starting dose based on body weight according to the manufacturer's instructions, which apparently is not sufficient to deal with inter-individual variability. In the present study a comprehensive analysis was performed on a rich data set to estimate the relative contribution of the factors that explain variability in early TRL exposure. With this population based PK approach an individualized TRL dosing strategy was developed in order to reach a predefined target exposure of TRL early after transplantation.

Patients and methods

Patients and immunosuppressive therapy

De novo kidney transplant recipients (n=31), aged between 18 and 70 years, were studied for one year following transplantation in the period September 2000 to March 2003. Recipient, donor and transplant characteristics as well as outcome parameters (acute rejection rate, patient and graft survival, renal function) are summarized in Table 1. Only recipients of a first kidney graft from a deceased or living (non HLA-identical) donor were included. Patients received quadruple immunosuppression, including induction therapy with basiliximab 20 mg before transplantation and on day 4, a fixed dose (500 mg twice daily) of mycophenolate mofetil (MMF), prednisolone (50 mg twice daily on the day of transplantation, rapidly tapered towards 5 mg once daily on day 22) and TRL. Patients re-

Table 1. Clinical characteristics.

Variable	Once daily (*n* = 16)	Twice daily (*n* = 15)	p-value
Recipient characteristics			
Age (mean ± SD)	43.9 ± 13.3	46.8 ± 12.0	0.53
Male sex (*n*)	12	12	0.74
Caucasian (*n*)	14	12	0.65
Native kidney disease			0.81
Glomerulonephritis	7	5	
Hereditary / congenital	3	5	
Hypertension	3	2	
DM	0	1	
Unknown	3	2	
Donor characteristics			
Age (mean ± SD)	47.4 ± 13.1	45.8 ± 14.3	0.75
Male sex (*n*)	13	6	0.03
DD-heart beating	6	7	0.72
DD-non heart beating	2	2	0.94
LRD	4	4	0.92
LURD	4	2	0.65
Transplant characteristics			
HLA-mismatch mean ± SD	2.81 ± 1.52	2.73 ± 2.25	
Class I	1.94 ± 1.06	1.87 ± 1.51	0.75
Class II	0.88 ± 0.72	0.87 ± 0.83	0.90
Cold ischemia time (h) DD only	18.7 ± 5.4	24.7 ± 7.8	0.09
Acute rejection 6 months (*n*)	0	2	0.14
Need for ATG (*n*)	0	2	0.14
Patient survival (*n*)			0.51
1 year	16	15	
2 years	16	15	
Death-censored Graft survival (*n*)			0.52
1 year	16	15	
2 years	15	14	
Nankivell clearance (mean ± SD)			0.39
Week 2	59 ± 30	53 ± 22	
Week 6	65 ± 15	60 ± 12	
Month 3	68 ± 11	65 ± 12	
Month 6	71 ± 12	65 ± 16	
Month 9	70 ± 12	64 ± 18	
Year 1	67 ± 14	62 ± 20	
Year 2	66 ± 14	60 ± 15	

DM, diabetes mellitus; DD, deceased donor; LRD, living related donor; LURD, living unrelated donor; HLA, human leukocyte antigen; ATG, anti-thymocyte globulin; SD, standard deviation.

ceived a TRL (Prograf®) daily dose of 0.2 mg/kg/day in either a once or twice daily regimen (Table 1). Therapeutic drug monitoring (TDM) for TRL twice daily (b.i.d.) regimen was aimed at an $AUC_{0\text{-}12h}$ of 210 μg×h/L in the first six weeks and subsequently lowered to 125 μg×h/L. For the once daily (o.d.) regimen these values were 420 μg×h/L and 250 μg×h/L respectively. From each patient written informed consent was obtained. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center.

Therapeutic drug monitoring

Therapeutic drug monitoring was performed on the basis of a limited sampling strategy (blood concentration at t=0, 2 and 3 h) and Bayesian estimation of the $AUC_{0\text{-}12h}$ using MW/Pharm version 3.5 (Mediware, Groningen, The Netherlands) as previously described [27]. Routine TDM samples (t=0, 2 and 3 h) were taken during the mornings of weeks 4, 8, 10, 17, 21 and 39 after transplantation. Additional samples were taken during the mornings of weeks 2, 6, 12, 26 and 52 with samples at t=0, 1, 2, 3, 4, 6 up to 12 hours after administration. These weeks were clustered for presentation purposes. As a matter of fact week 2 is the first rich sampling moment performed in the first 3 weeks after transplantation with a median on day 10 after transplantation and the first day as early as day 6. In order to reach a steady state, TRL exposure was determined several days after transplantation. This did not rule out trough level monitoring in the first week after transplantation to avoid extreme low and high exposure. All patients were sampled on 11 occasions, with the exception of 5 patients who were sampled less frequently (ranging from 4 to 10 occasions). TRL blood concentrations were determined in whole blood by micro particle enzyme immunoassay (MEIA, Abbott laboratories, Abbott Park, IL, USA). Assay inter-day variation, derived from routine measurements, was 20% (5 μg/L), 15% (11 μg/L) and 12% (22 μg/L). The linear range of the assay was up to 30 μg/L. Levels higher than 25 μg/L were diluted according to the manufacturer's instructions.

Genotyping assays

DNA was isolated from EDTA-blood. Primers and probes used in the Taqman based genotyping assays, as well as primers and sequences used in the Pyrosequence assays are listed in Table 2. *ABCB1* C1236T, T3435C and G2677T were determined with TaqMan 7500 (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands) with custom designed assays, according to the manufacturer's protocol. *ABCB1* T-129C, *CYP3A5* *3 and *6, *CYP3A4**1B and *PXR (NR1I2)* C-25385T, A-24381C, G-24113A, A+252G, A+7635G were determined with Pyrosequencer 96MA (Isogen, IJsselstein, The Netherlands). In short, PCR reactions contained 10 nanogram of DNA, and 5 pmol of each PCR primer (listed in table 2) in a total volume of 12 microliters. Cycle conditions were: initial denaturation for 15 minutes at 95°C, 35 cycles of 95°C-55°C-72°C each for 30 seconds, ended by 10 minutes at 72°C. The pyrosequence reactions were performed according to the manufacturer's protocol. The sequence used for analysis and the calculated dispensation order for each SNP are listed in Table 2. The nucleotides shown in lower case are negative controls, which were not incorporated in the target DNA and consequently did not appear in the pyrogram. As quality control, 5% of samples were genotyped in duplicate. In addition, negative controls (water) were used. No inconsistencies were observed. All allele frequencies were in Hardy-Weinberg equilibrium. Table 5 presents the genotype distribution in the overall genotyped population.

Table 2. Primers and Probes for TaqMan and Pyrosequence analysis.

SNP	Target[a]	Sequence 5'-3	Modification[b]
ABCB1 C1236T	PCR-f	CACCGTCTGCCCACTCT	
	PCR-r	GTGTCTGTGAATTGCCTTGAAGTTT	
	Probe-T	TTCAGGTTCAGACCCTT	VIC
	Probe-C	CAGGTTCAGGCCCTT	FAM
ABCB1 G2677T	PCR-f	CTTAGAGCATAGTAAGCAGTAGGGAGT	
	PCR-r	GAAATGAAAATGTTGTCTGGACAAGCA	
	Probe-G	TTCCCAGCACCTTC	VIC
	Probe-T	TTCCCAGAACCTTC	FAM
ABCB1 T3435C	PCR-f	ATGTATGTTGGCCTCCTTTGCT	
	PCR-r	GCCGGGTGGTGTCACA	
	Probe-T	CCCTCACAATCTCT	VIC
	Probe-C	CCCTCACGATCTCT	FAM
Pyrosequence			
ABCB1 T-129C	PCR-f	TCGAAGTTTTTATCCCA	Biotine
	PCR-r	CCTCCTGGAAATTCAACCTGTT	
	Sequence primer	TACTCCGACTTTAGTGGAAAGACC	
	Target Sequence	CTG/ACTCGAATGAG	
CYP3A5*3	PCR-f	CTGCCTTCAATTTTTCACT	
	PCR-r	TATGTTATGTAATCCATACCCC	Biotine
	Sequence primer	AGAGCTCTTTTGTCTTTCA	
	Target Sequence	A/GTATCTC	
CYP3A4*1B	PCR-f	CAGCCATAGAGACAAGGGC	
	PCR-r	GAAGAGGCTTCTCCACCTT	Biotine
	Sequence primer	CCATAGAGACAAGGGCA	
	Target Sequence	A/GGAGAGAGG	
CYP3A5*6	PCR-f	TCTTTGGGGCCTACAGCATG	
	PCR-r	AAAGAAATAATAGCCCACATACTTATTGAGAG	Biotine
	Sequence primer	AGAAACCAAATTTTAGGAA	
	Target Sequence	CTTC/TTTAG	
PXR C-25385T	PCR-f	GTGGTCATTTTTTGGCAATCCC	
	PCR-r	AGCCTCTGGCAACAGTAAAGCA	Biotine
	Sequence primer	TTGGCAATCCCAGGT	
	Target Sequence	TC/TTCTTTTCTACCTGTT	
PXR A-24381C	PCR-f	AGTGGGAATCTCGGCCTCA	
	PCR-r	CTGGGGTCCACTTTGAACAATC	Biotine
	Sequence primer	GCTAATACTCCTGTCCTGAA	
	Target Sequence	A/CAAGGCAGCGGCTCCTTG	
PXR G-24113A	PCR-f	GAATCATGTTGGCCTTGCTGC	
	PCR-r	GCATCAGTAATGGGGCTCAAC	Biotine
	Sequence primer	TCTCCTCATTTCTAGGGT	
	Target Sequence	C/TCACCCTAG	
PXR A+252G	PCR-f	TGCAAGGGCTTTTTCAGGTAGAGT	
	PCR-r	TGAACCTGGGGGATAGGTCAAG	Biotine
	Sequence primer	ACTGACCCACTGGGTAA	
	Target Sequence	CA/GTCTCAGGGC	
PXR A+7635G	PCR-f	AGCCATCCTCCCTCTTC	Biotine
	PCR-r	CAGCAGCCATCCCATAATC	
	Sequence primer	CATAATCCAGAAGTTGGG	
	Target Sequence	GGC/TGAGAGGAA	

[a] f = forward orientated, r = reverse orientated. [b] VIC and FAM are fluorescent dyes, biotine is necessary to obtain single stranded DNA. ABC, ATP-binding cassette; CYP, cytochrome P450; PXR, pregnane X receptor; PCR, polymerase chain reaction.

Population pharmacokinetic analysis

The PK of TRL were analyzed by non-linear mixed effects modelling (NONMEM) [28]. Mixed effects models consists of a structural model, describing the relationship between dose and concentration in terms of structural PK parameters (i.e. clearance (CL), volume of distribution (V)), and a stochastic model, describing the random variability in the structural model parameters. For population PK modelling these random-effects are the expression of inter-individual and inter-occasion variability. Inter-individual variability describes the random variability of structural parameters within the population, whereas inter-occasion variability describes the variability of an individual parameter value from one occasion to another. The second level of stochastic-effects, σ^2, describes the variability of the difference between observed and predicted responses. This residual error includes among other factors model misspecification, intra-individual variability and measurement error. In the mixed-effects modelling approach, structural and stochastic parameters are simultaneously estimated by fitting the model to the data. In this respect the following parameters were estimated: PK parameters, variance and covariance (ω^2) of each individual specific parameter value (η) and variance (σ^2) of the residual error. As a result, individual *post hoc* estimates of parameters associated with inter-individual variability and inter-occasion variability could be obtained.

STRUCTURAL MODEL. The PK of TRL was fitted to linear compartmental models. As in the present analysis only data after oral administration were available, the absolute oral bioavailability could not be identified. Therefore, the value for the oral bioavailability was fixed to 23%, as previously described [29] and used in the clinically applied TDM model [27].

RANDOM EFFECTS. Inter-individual variability and inter-occasion variability were described assuming a log normal distribution with the following equation:

$$PKj = TVPK \times e^{\eta jPK}$$

in which PKj is the PK parameter for the jth individual and ηjPK is the difference between the individual specific parameter and the population value. TVPK is the population value of the PK parameter and the difference of the logarithm between the individual value of subject j and the population mean (ηjPK) is normally distributed with a mean of zero and variance ω^2_{PK}. The residual error was assumed to be proportional to the predicted concentration:

$$Cij = Cpredij \times (1 + \varepsilon ij)$$

in which Cij is the ith observation for the jth individual, Cpredij is the concentration of TRL in the blood predicted by the PK model, and εij (difference between Cij and Cpredij) is a normally distributed random variable with mean zero and variance σ^2.

COVARIATE ANALYSIS. The following covariates were selected on the basis of their known or theoretical relationships with TRL PK: hematocrit, serum albumin concentration, serum cholesterol (LDL, HDL) concentration, prednisolone dose, TRL dose regimen (once or twice daily dosing), polymorphisms in *ABCB1*, *CYP3A5*, *CYP3A4*, and *PXR*, and demographic factors such as body weight, age and gender. Only covariates with a clear visual

Table 3. Population pharmacokinetic parameters for TRL obtained from the bootstrap of the final model. This table shows the mean and coefficient of variation of the PK parameter estimates as well as the median and percentiles of these estimates. The variability concerns the actual random variability in the PK parameter relative to the population mean value.

PK Parameter	Mean value	variability	CV (%)	median	Percentiles 2.5-97.5 (%)
CL CYP3A5*3*3 (L/h)	3.7		8.2	3.8	3.1-4.3
CL CYP3A5*1*3 (L/h)	5.5		9.8	5.5	4.4-6.6
F (fixed)	0.23				
F (pred > 10 mg)	-15% [a]		-30		
V_c (once daily dosing) (L)	61		11	61	48-74
V_c (twice daily dosing) (L)	42		10	42	35-51
Q (L/h)	10		10	10	8-12
V_p (L)	$=V_c$ [b]				
k_a (once daily dosing) (h^{-1})	3.7		23	3.7	2.2-5.1
k_a (twice daily dosing) (h^{-1})	1.6		14	1.6	1.2-2.1
D_{50} (mg)	25		30	24	14-42
IIV CL (ω^2)	0.038	19%	32	0.037	0.016-0.064
IIV V_c (ω^2)	0.080	28%	31	0.081	0.034-0.129
IOV F (ω^2)	0.047	22%	13	0.047	0.036-0.058
Residual var. (σ^2)	0.051	23%	6	0.051	0.044-0.057

CL, clearance; F, bioavailability; V_c, central volume of distribution; Q, inter-compartmental clearance; V_p, peripheral volume of distribution; k_a, absorption rate constant; D_{50}, dose with half minimal bioavailability; pred, prednisolone dose; CV, coefficient of variation; IOV, inter occasion variability; IIV, interindividual variability. Var, variability. [a] This means a 15% lower value for TRL bioavailability. [b] V_p is equal to V_c due to imprecise parameter estimation according to the bootstrap analysis.

relationship between the random effects in the base model (i.e. the model without covariates) and the covariate values were formally tested. Subsequently, the selected covariate relationships were evaluated by a forward inclusion and a backward deletion procedure [30]. A covariate effect was only maintained in the model if the inclusion resulted in a reduction in the random variability and in an improved model fit.

COMPUTATION. Non-Linear-Mixed-Effects-Modelling (NONMEM, version VI, Icon Development Solutions, Ellicott City, Maryland, USA) was used for modelling TRL PK. Modelling results were analyzed using the statistical software package S-Plus® for Windows (version 6.2 Professional, Insightful Corp., Seattle, USA). A convergence criterion of 3 significant digits in the parameter estimates was used. For model comparisons, the obtained minimum value of the objective function (MVOF) defined as minus twice the log-likelihood was used. First order conditional estimation (FOCE) with interaction was used throughout the modelling process. The modelling process was guided by statistical and visual checks (i.e. diagnostic 'goodness of fit' plots).

A model parameter was retained in the model when the difference in the minimum value of the objective function (minus twice the log likelihood) from the base model was at least 6.63 points. This compares to a Type I error of 1% under the assumption that this difference is χ^2 distributed with 1 degree of freedom. However, it is known that the NONMEM FOCE method produces only an approximation to the maximum likelihood assumptions and that the null hypothesis will be rejected more frequently than the nominal Type I error value [31,32]. Therefore, a randomization test (also called *re*-sampling or permutation) was performed to estimate the true distribution under the null hypothesis (i.e. the probability of observing the covariate effect by chance alone). This allows deriving the nominal difference in the objective function to reject the null hypothesis of no difference between the model with and without the covariate of interest with a specific Type I error rate. The randomization test involves fitting the model without and with the covariate effect to randomized data where the covariate was randomly allocated to subjects breaking any association between the covariate and the PK parameter of interest. This procedure was repeated 1,000 times generating a distribution of differences in the minimum value of the

Table 4. Covariate table. Listed in this table are the significant covariates improving model fit together with the effects on the observed variability. The forward inclusion and backward deletion procedure are displayed according to the procedure explained in the results section.

COVARIATE TESTED	MVOF	ΔOF[a]	IIV CL (%)	Expl var. CL (%)	IIV V_c (%)	Expl Var V_c (%)
BASE MODEL	5969		29		29	
+CYP3A5*1 (CL)	5951	-18	20	9	29	
+PXR A+7635G (CL)	5961	-8	25	4	30	
+ABCB1 T-129C (CL)	5965	-4[b]	26	3	29	
+Ht (allometric) (V_c)	5965	-4[b]	29		27	2
Forward inclusion						
BASE	5969		29		29	
BASE+CYP3A5	5951	-18	20	9	29	
Backward deletion						
FINAL MODEL	5951		20		29	
- PRED	5961	+10	19		28	
- PRED-CYP3A5	5981	+30	27		28	
- PRED-CYP3A5-GRP	6003	+52	27		30	
- PRED-CYP3A5-GRP-DDOSE	6059	+108	35		36	

CYP, cytochrome P450; PXR, pregnane X receptor; ABC, ATP-binding cassette; DDOSE, daily dose TRL; Ht, hematocrit; PRED, daily dose prednisolone; GRP, once and twice daily dose group; MVOF, minimum value of the objective function; ΔOF, change in MVOF; IIV, inter-individual variability; Expl.Var, explained variability; CL, clearance, V_c, central volume of distribution. [a]ΔOF: Decrease in the minimum value of the objective function compared to the base model or increase compared to final model. [b]not significant (P>0.01). It is significant with 95% confidence p<0.05 (ΔOF>3.84).

objective function (MVOF) between these models. The 99th percentile of this distribution reflects the difference in the MVOF for a Type I error rate of 1%.

VISUAL PREDICTIVE CHECK. The model prediction was evaluated using a Visual Predictive Check (VPC), which evaluates whether the identified model would be able to predict the observed variability for 80% of the population in the PK data that was used for model identification. Therefore, the PK of each individual using its individual specific dosing history and covariate values was simulated at least 200 times by means of a Monte Carlo simulation, in which random samples were drawn from the identified distributions for inter-individual variability, inter-occasion variability and residual variability. The distribution (median and 10th and 90th percentiles) of the simulated concentration-time courses was compared with the distribution of the observed values in the original data set. Differences and overlap of the simulated and original distributions indicated the accuracy of the identified model.

BOOTSTRAP. A bootstrap analysis was performed to assess the precision of the PK parameter estimates. The observed data set was re-sampled with replacement in order to generate a new data set with the same size and population characteristics, such as the number of patients per genotype, as the original set. This procedure was repeated 1,000 times to generate a distribution of the PK parameters with a mean and coefficient of variation as well as the median and 2.5^{th} and 97.5^{th} percentiles. Fitting the same re-sampled data set with the base model (i.e. model without covariate effect) and calculating the difference in MVOF between the base and covariate model allows calculating the power of the study to estimate a covariate retrospectively. The power is defined as the number of times that the difference in MVOF is greater than the difference associated with a type I error rate of 1% derived from the randomization test.

Results

Structural model

The PK of TRL was best described by a two compartment model with first-order absorption and first-order elimination from the central compartment. Random effects parameters were identified for the inter-individual variability in TRL clearance (CL) and the volume of the central compartment. Moreover, the variability between the occasions at which PK samples were collected was described with a random effect on the (fixed) bioavailability term (F). As a next step, the random effects were evaluated for structural relationships with dose, dosing frequency and time, to obtain a model with unbiased and randomly distributed random effects for the covariate analysis. A relationship between dose and clearance was observed, showing an increase in apparent clearance with increasing dose according to $F = 0.23 \times [1 - (\text{daily dose}/(D_{50} + \text{daily dose}))]$. D_{50} is the estimated dose with half minimal bioavailability (Table 3). Incorporating this relationship improved the model fit considerably in terms of objective function and goodness of fit plots. Secondly, the random effect parameters were not randomly distributed due to an overall decrease in apparent clearance (CL/F) in the first 6 weeks after transplantation. It appeared that co-administrating prednisolone in a dose over 10 mg/day was related to a 15% lower TRL bioavailability compared to lower doses. A final source of bias in the analysis arose from

the fact that once daily TRL dosing was clearly associated with an increased absorption rate as well as an increase in volume of distribution (Table 3). The model including all of the above described relationships was considered the base model for studying covariate effects.

Covariate model

The data showed considerable inter-individual variability (29%) and inter-occasion variability (22%) in CL/F with values ranging from 8-78 L/h. The variability in TRL clearance could be explained by genetic factors as two subpopulations with different values for TRL clearance were observed. These populations could be related to a genetic polymorphism (SNP) in CYP3A5 (Figure 1). Specifically, in the CYP*3/*3 genotype CL=3.7±0.3 L/h whereas in the CYP3A5*1/*3 genotype clearance was: 5.5±0.5L/h. Thus, the CYP3A5*1/*3 genotype was associated with higher TRL clearance, hence lower exposure in terms of AUC (Figure 2). This SNP accounted for 9% of the inter-individual variability in TRL clearance (Table 4). Relative to the observed variability in apparent clearance of 29% this genetic factor explained 30% of the inter-individual variability in TRL exposure. The estimated difference in clearance is significant, since the decrease in MVOF from the model without this covariate effect is 18 points (Table 4), which is considerably greater compared to the difference of 5.42 required for a significant difference with a type I error of 1%. In addition, a retrospective power of 99% was found, indicating that the study was sufficiently powered to estimate the difference in clearance.

The second relevant genetic factor was PXR A+7635G. In A-allele carriers CL=3.9±0.3 *versus* CL=5.4±0.5L/h in the GG genotype. Thus the PXR7635 GG SNP was also associated with higher TRL clearance, but explained only 4% of the inter-individual variability

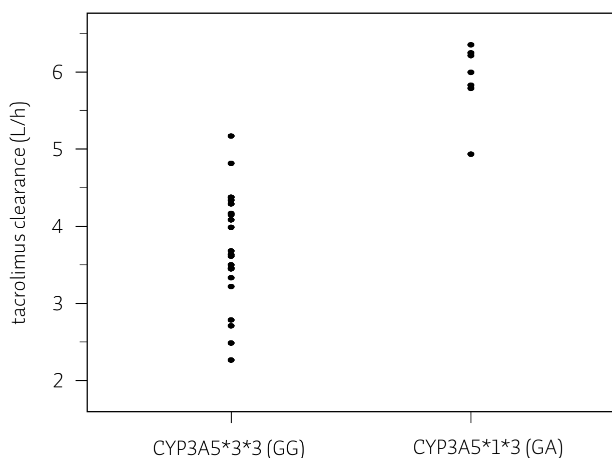

Figure 1. The relationship between CYP3A5 genotype and tacrolimus clearance.

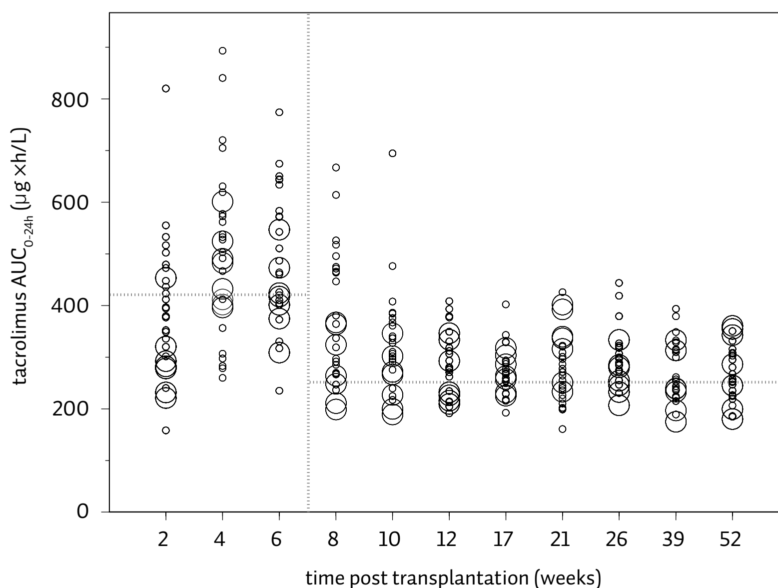

Figure 2. AUC0-24 hours *versus* time post transplantation, small circles are patients with the CYP3A5*3*3 genotype, large circles represent CYP3A5*1*3. The dotted lines are the target AUC0-24 hours in the first 6 weeks (left, top) and the target value beyond the first 6 weeks (right, bottom). Note that week 2 is a grouping category including exposure measurements as soon as day 6 after transplantation.

in TRL clearance (Table 4). Three out of five individuals with the PXR A+7635G GG genotype displayed an overlap with 3 (out of 7) individuals carrying the CYP3A5*1/*3 genotype. Moreover, an association between the CT genotype in the ABCB1 gene promoter SNP T-129C was associated with a decreased TRL clearance (Table 4). This SNP accounted for 3% of the variability in TRL clearance with this limited number of observations (Table 5). The remainder of the SNPs in the ABCB1-gene (encoding the P-glycoprotein transporter) was studied together by means of the amount of functional alleles or gene-dose effect, but a correlation with TRL clearance was not found. The other selected PXR genotypes (PXR C-25385T, PXR A-24381C, PXR G-24113A, PXR A+252G) did not have a relationship with TRL clearance either.

Another interesting factor is hematocrit which displayed an allometric relationship with central volume of distribution (V_c) (i.e. $V_c=61\times(Ht/0.37)^{-0.5}$), indicating a decreasing V_c upon rising hematocrit. This relationship was clearly present during the visual covariate analysis and caused a decrease in objective function, which was not considered strong enough to be incorporated into the model (Table 4).

Interestingly, body weight (43-109 kg, mean and median 75 kg) could not be identified as a covariate on neither CL/F nor V_c/F. The relationship between dose and weight introduced by weight-based dosing disappeared by applying TDM. Figure 3A demonstrates the absence of a relationship between body weight and TRL clearance. Yet, a relationship between body weight and the difference between observed AUC and target AUC (AUC

observed - target AUC) was observed (Figure 3B). This plot visualizes the relationship between the initial TRL dose and achieving target exposure. The left plot has been obtained from the data. The plot on the right is the same plot but scaled to a dose of 0.2 mg/kg/day. Scaling was done to correct for the fact that obese patients were dosed on lean body weight and in most cases received a lower body weight based TRL dose. Figure 3B indicates that underexposure could be related to low bodyweight and overexposure to higher bodyweight. The covariates serum albumin concentration, cholesterol (HDL, LDL, total), age and gender, were found not to have a relationship with TRL PK.
Finally, the results of the backward deletion procedure are presented in Table 4, where the effect of sequentially deleting the relevant covariates prednisolone daily dose, CYP3A5 genotype, once and twice daily TRL dosing and daily TRL dose from the model is shown. The final model was found to adequately predict the observed trend and variability in the TRL concentrations according to the visual predictive check (Figure 4).

Discussion

In the present study a comprehensive analysis was performed to estimate the contribution of a wide range of factors involved in the variability of early TRL exposure. The aim was to identify covariates relevant for individualized dosing. The three most relevant factors identified were, in rank order of importance, CYP3A5*1/*3, PXR A+7635G GG and prednisolone co-medication in a dose over 10 mg per day. The clear effect of the SNP in CYP3A5 regardless of the patient's body weight underscores the importance of an individualized initial dosing strategy in this population of adult kidney transplant recipients. In the current analysis patients with the CYP3A5*1/*3 genotype required a 1.5 times higher fixed initial dose compared to CYP3A5*3/*3 in order to reach adequate TRL target exposure early after transplantation.
In this study no subjects with CYP3A5*1/*1 genotype were found. Since the *1 allele is associated with increased CYP3A5 function, one would expect a higher TRL dose for the *1/*1 genotype as well [20]. Earlier studies have documented the impact of polymorphisms in genes encoding CYP3A4, CYP3A5 and ABCB1 (P-glycoprotein) on TRL PK, but in the large majority only in relation to (dose-corrected) trough levels [13,15,33]. Unfortunately, trough levels have repeatedly been shown to correlate poorly with the systemic exposure measured in terms of AUC [27,34]. Indeed, the use of trough levels to adjust the TRL dose in clinical practice will introduce variability in the actual TRL exposure, since we observed a trough level range of 3 to 20 µg/L when dosing twice daily aiming at a target AUC of 210 µg×h/L (range 190 to 230 µg/L).
The population analysis methodology used in the present study differentiates between structural variability (within an individual) and random variability (between individuals). In contrast to non-population based approaches this results in greater statistical power to identify a covariate effect, because both sources of information are used instead of one. When analyzing multiple observations per subject one is able to compensate for the small number of individuals. This is supported by the retrospective power analysis, which shows that the power was 99% to identify a difference in TRL clearance of 47% between carriers of the CYP3A5*1 and the CYP3A5*3 genotype.

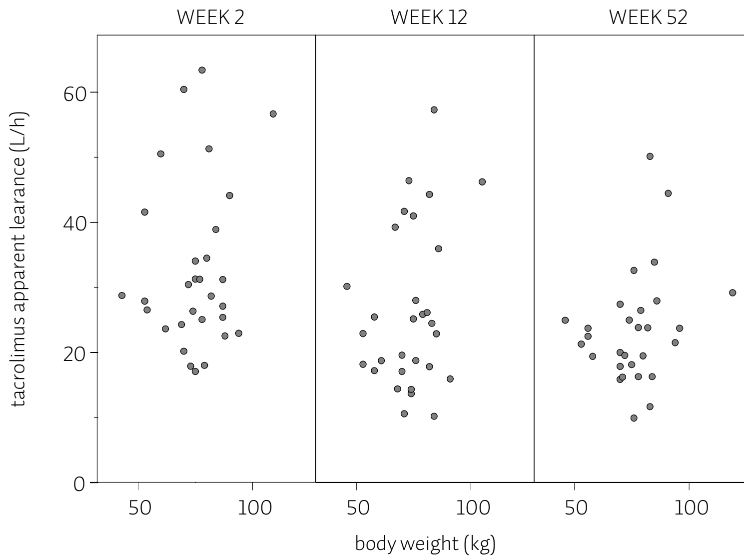

Figure 3A. The relationship of TRL apparent clearance with body weight plotted on 3 occasions (week 2, 12 and 52) after transplantation.

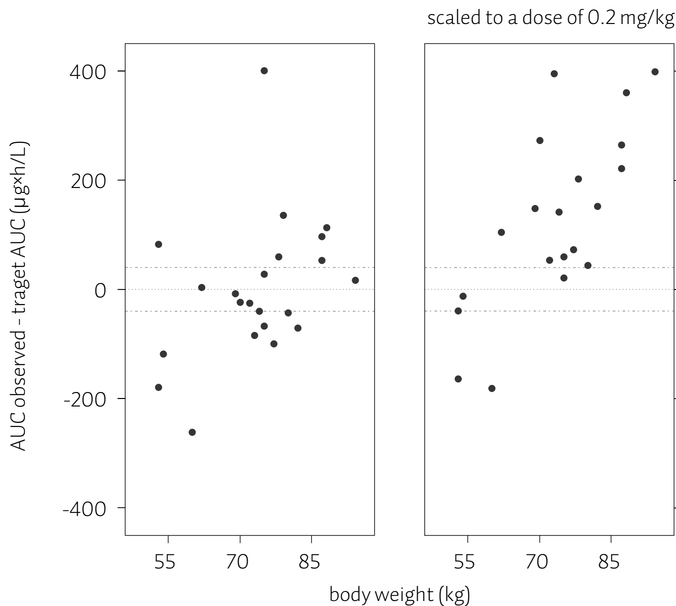

Figure 3B. $AUC_{0\text{-}24h}$ observed minus target $AUC_{0\text{-}24h}$ on the *first* exposure measurement post transplantation (CYP3A5*3*3 only) on the left. In the right plot the data are scaled to the theoretical dose of 0.2 mg/kg/day.

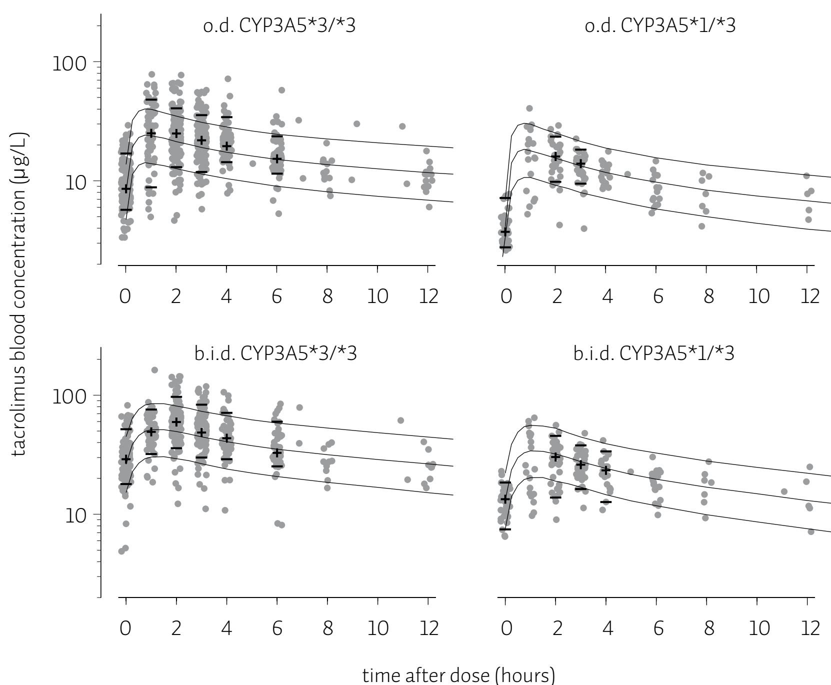

Figure 4. The visual predictive check with the 80% prediction interval (area between the outer solid lines) depicted for the genotype groups CYP3A5*1/*3 and CYP3A5*3/*3 and the once and twice daily dose groups. The middle solid line represents the median of the model prediction. The observed concentrations are shown as closed symbols, whereas the median of observed concentrations per time point are shown with the cross (+) symbol. The horizontal lines indicate the 10th and 90th percentile of the observed data. In case the PK analysis indicated linear PK, that would allow scaling the TRL doses to the median of the dose, 7 mg for the o.d. and 5 mg for the b.i.d group, as different doses are administered to patients due to TDM. o.d.: once daily dosing, b.i.d.: twice daily dosing.

There are two possible confounding factors relevant for this analysis. First, the identified CYP3A5 genotype effect could potentially be influenced by the immunoassay used to determine TRL blood concentrations. It is known that this assay displays cross-reactivity of TRL metabolites, which limits assay performance [35,36]. Patients with the CYP3A5*1 genotype have an increased TRL metabolism resulting in a higher metabolite fraction in the blood samples obtained during routine monitoring. In theory, these samples could be biased more by the cross-reactivity due to the higher metabolite fraction. However, it is not likely that this interfered with the obtained results as it has been shown by Moreton et al that CYP3A5 genotype differences do not influence the blood concentration determination [37]. And, furthermore, if this would have interfered with the obtained results, this would have inflated the result. The CYP3A5*1 group would then in fact have lower actual blood levels, hence higher clearance than already demonstrated. A second important question is whether we would have to consider a circadian variation, also called chronopharmacokinetics, in TRL metabolism. This is currently under debate in literature [38-42]. No difference in daytime versus night time AUC was observed in several studies with oral TRL

Table 5. Genotype distribution in the study population (*n* = 31).

SNP	Frequency			
ABCB1 T3435C	T/T 7 (23%)	C/T 14 (47%)	C/C 9 (30%)	1 UG
ABCB1 G2677T	G/G 10 (33%)	G/T 16 (53%)	T/T 4 (13%)	1 UG
ABCB1 C1236T	C/C 11 (37%)	C/T 14 (47%)	T/T 5 (17%)	1 UG
ABCB1 T-129C	T/T 27 (90%)	C/T 3 (10%)	C/C 0	1 UG
CYP3A4*1B	A/A 26 (87%)	G/A 4 (13%)	G/G 0	1 UG
CYP3A5*3	G/G 23 (77%)	A/G 7 (23%)	A/A 0	1 UG
CYP3A5*6	C/C 30 (100%)	C/T 0	T/T 0	1 UG
PXR C-25385T	C/C 8 (27%)	C/T 18 (60%)	T/T 4 (13%)	1 UG
PXR A-24381C	A/A 7 (23%)	A/C 19 (63%)	C/C 4 (13%)	1 UG
PXR G-24113A	G/G 8 (28%)	G/A 17 (59%)	A/A 4 (14%)	2 UG
PXR A+252G	A/A 11 (37%)	A/G 13 (43%)	G/G 6 (20%)	1 UG
PXR A+7635G	T/T 11 (38%)	C/T 13 (45%)	C/C 5 (17%)	2 UG

SNP, single nucleotide polymorphism; ABC, ATP-binding cassette; CYP, cytochrome P450; PXR, pregnane X receptor; UG, unsuccessfully genotyped (1 individual in particular). Frequency determined on successfully genotyped individuals.

[38,39,41], while a slower and delayed absorption was identified in one of these studies [41]. Interestingly, two studies pointed out that night-time TRL administration resulted in significantly lower AUC and C_{max} [42,43], while intravenous TRL did result in a slightly lower clearance and consequently higher AUC at night compared to morning administration [40]. It is thought that the absorption process in terms of gastric emptying time and perfusion possibly influenced by an evening meal is crucial in the observations [3,42]. The circadian effect itself is therefore contestable. In case the effect exists it does not seem to be a large effect. Moreover, it is unlikely that this would have influenced our findings which are based on morning administration of TRL in a once or twice daily fashion.

A new polymorphism (PXR A+7635G) in the gene encoding for the pregnane X receptor (PXR) was identified which explained 3.5% of the variability in TRL exposure. However, the impact of this polymorphism was relatively low in comparison to the already discussed polymorphism in CYP3A5 which explained 9% of the inter-individual variability in apparent clearance (CL/F) (or 30% of the inter-individual variability in CL/F). Importantly, activation of the PXR is one of the determinants involved in the (tissue specific) expression of both CYP3A and ABCB1. Therefore, the role of PXR can be especially relevant in the first weeks or months after organ transplantation, when usually high prednisolone doses are prescribed. A maintenance dose of 5-10 mg/day is still considered to be pharmacologically active resulting in a variable continuation of PXR activation. In addition, other endogenous (cortisol) or unknown factors in transplant recipients may be respon-

sible for activation of the PXR. This is supported by the observation, that a subgroup of our patients displayed a decrease in CL/F within the first 6 weeks after transplantation that could not be related to the SNPs studied in the PXR gene.
The present model was improved by including the effect of prednisolone on TRL bioavailability, which was a relative term due to the absence of intravenous TRL data. A concomitant prednisolone dose over 10 mg/day resulted in a 15% lower TRL bioavailability compared to a concomitant prednisolone dose of 10 mg/day or lower. A limitation to the present study was the absence of the effect of treating acute rejection episodes with high dose steroids (Solu-Medrol) [11]. Neither could we determine the effect of a low dose (less than 10 mg/day) or a steroid free regimen on TRL apparent clearance [10].
TRL absorption and distribution kinetics differed when considering the once daily and twice daily dosing regimens. A higher absorption rate and higher volume of distribution were observed for the once daily TRL dosing group. However, this is not clinically relevant since the TRL dosing interval did not appear to have an effect on TRL exposure. Recently a modified release formulation of tacrolimus has been introduced in order to improve patient compliance [44,45]. The clinical and pharmacokinetic data obtained in the present study indicate that once daily dosing could also be an option with the conventional formulation.
The observed relationship between TRL dose and clearance could, at least partly, be the result of an interaction between prednisolone and TRL, explained by a decreased inductive effect on TRL clearance following steroid taper. The relationship between TRL dose and clearance is however most likely the consequence of patient selection through the use of the strict adherence to the defined TDM protocol. According to the protocol, the patients with high TRL blood levels (i.e. with a lower clearance) were titrated to receive lower doses and *vice versa*. Therefore the apparent relationship between CL/F and dose reflects the study design, i.e. is the result of adjusting the dose on the basis of a predefined target. This conclusion is supported by the observation that before TDM was started, no relationship between dose and CL/F was found. A recent simulation study has documented a similar effect for carbamazepine [46].
High dose TRL in combination with a low hematocrit resulted in a non-linear PK behaviour which has been explained by saturation of binding capacity to red blood cells [7,25]. In the present analysis, with hematocrit values in the range of 0.26 to 0.52, such a relationship could not be clearly identified. It has been shown that hematocrit influences the TRL blood level determinations with an immunoassay [47-49]. However, as this especially concerns hematocrit values of 0.25 and lower this does not form a major issue in this study. The relationship between hematocrit and TRL PK has been described before [7,25]. Low hematocrit values resulted in a higher apparent volume of distribution, reflecting more unbound TRL available for distribution in peripheral tissues. The observed non-linearity may have relevant clinical implications (delayed graft function, liver- and neurotoxicity) especially in the first weeks after organ transplantation, where low hematocrit is accompanied by high TRL doses. Other potentially relevant factors that could contribute to observed variability in TRL PK, such as diarrhea [50,51] and non-compliance, could not be quantified in this study.

TRL is a low clearance drug and in adult transplant recipients, body weight was not related to either central volume of distribution or TRL clearance (Figure 3A). Our data (Figures 2 and 3B) indicate that a predefined target can be reached faster using a genotype-based instead of a bodyweight-based initial dosing regimen. In the present study the TRL dose was adjusted to lean body mass by protocol. As a result, patients with a body mass index over 25 on average received 0.15 mg/kg body weight per day. Taking this into account the relationship strengthened (Figure 3B), indicating that without this intervention these patients would have received a starting dose which was far too high. A fixed standard dose of 14 mg/day (corresponding to 0.2 mg/kg/day for a 70 kg person) to reach the defined AUC could be a better therapeutic approach for patients with the CYP3A5*3/*3 genotype compared to the currently applied body weight based regimen. Moreover, a fixed daily dose of 20 mg (10 mg b.i.d.) would be more appropriate in patients with the CYP3A5*1/*3 genotype due to the 1.5 times higher apparent clearance. Clearly, this strategy warrants validation in a prospective study which includes larger numbers of renal transplant recipients
Despite the well documented high inter patient variability in TRL pharmacokinetics, bodyweight is still the only variable that drives the TRL starting dose at the time of transplantation. The findings of the present study suggest that a genotype-based dosing strategy in combination with TDM, may be a novel and superior approach to optimize initial exposure in adult kidney transplant recipients. Justification for this approach is the current trend towards early CNI minimization protocols, since both acute rejection and acute CNI-induced nephrotoxicity have been identified as the major risk factors for chronic allograft nephropathy [2].

Conclusions

Three covariates were found to contribute to individualized TRL dosing in order to optimize early TRL exposure in kidney transplant patients. Adult CYP3A5*1 allele carriers have a higher TRL clearance compared to CYP3A5*3 allele carriers. These individuals, therefore, should receive higher TRL fixed starting doses since TRL starting doses should not be adjusted to patient's body weight. Moreover, the Pregane-X-Receptor A+7635G GG genotype as well as concomitant prednisolone administration in a dose over 10mg/day are associated with increased TRL metabolism.

1. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; 357(25):2562-2575.
2. Solez K, Vincenti F, Filo RS. Histopathologic findings from 2-year protocol biopsies from a U.S. multicenter kidney transplant trial comparing tarolimus versus cyclosporine: a report of the FK506 Kidney Transplant Study Group. *Transplantation* 1998; 66(12):1736-1740.
3. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet* 2004; 43(10):623-653.
4. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V *et al.* Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet* 1995; 29(6):404-430.
5. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Changes in tacrolimus distribution in blood and plasma protein binding following liver transplantation. *Ther Drug Monit* 2004; 26(5):506-515.
6. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *Br J Clin Pharmacol* 2004; 57(3):298-309.
7. Chow FS, Piekoszewski W, Jusko WJ. Effect of hematocrit and albumin concentration on hepatic clearance of tacrolimus (FK506) during rabbit liver perfusion. *Drug Metab Dispos* 1997; 25(5):610-616.
8. Jusko WJ, Piekoszewski W, Klintmalm GB, Shaefer MS, Hebert MF, Piergies AA *et al.* Pharmacokinetics of tacrolimus in liver transplant patients. *Clin Pharmacol Ther* 1995; 57(3):281-290.
9. Anglicheau D, Flamant M, Schlageter MH, Martinez F, Cassinat B, Beaune P *et al.* Pharmacokinetic interaction between corticosteroids and tacrolimus after renal transplantation. *Nephrol Dial Transplant* 2003; 18(11):2409-2414.
10. Hesselink DA, Ngyuen H, Wabbijn M, Gregoor PJ, Steyerberg EW *et al.* Tacrolimus dose requirement in renal transplant recipients is significantly higher when used in combination with corticosteroids. *Br J Clin Pharmacol* 2003; 56(3):327-330.
11. Shimada T, Terada A, Yokogawa K, Kaneko H, Nomura M, Kaji K *et al.* Lowered blood concentration of tacrolimus and its recovery with changes in expression of CYP3A and P-glycoprotein after high-dose steroid therapy. *Transplantation* 2002; 74(10):1419-1424.
12. Christians U, Jacobsen W, Benet LZ, Lampen A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clin Pharmacokinet* 2002; 41(11):813-851.
13. Hesselink DA, van Schaik RH, van der Heiden I, van der Werf M, Gregoor PJ, Lindemans J *et al.* Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 2003; 74(3):245-254.
14. Kamdem LK, Streit F, Zanger UM, Brockmoller J, Oellerich M, Armstrong VW *et al.* Contribution of CYP3A5 to the in vitro hepatic clearance of tacrolimus. *Clin Chem* 2005; 51(8):1374-1381.
15. MacPhee IA, Fredericks S, Mohamed M, Moreton M, Carter ND, Johnston A *et al.* Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in whites and South Asians. *Transplantation* 2005; 79(4):499-502.
16. Thervet E, Anglicheau D, King B, Schlageter MH, Cassinat B, Beaune P *et al.* Impact of cytochrome p450 3A5 genetic polymorphism on tacrolimus doses and concentration-to-dose ratio in renal transplant recipients. *Transplantation* 2003; 76(8):1233-1235.
17. van Schaik RH, van der Heiden I, van den Anker JN, Lindemans J. CYP3A5 variant allele frequencies in Dutch Caucasians. *Clin Chem* 2002; 48(10):1668-1671.
18. Szekeres T, Haushofer A. Clinical pharmacogenetics of immunosuppressive drugs in organ transplantation. *Pharmacogenomics* 2005; 6(2):163-168.
19. Thervet E, Legendre C, Beaune P, Anglicheau D. Cytochrome P450 3A polymorphisms and immunosuppressive drugs. *Pharmacogenomics* 2005; 6(1):37-47.
20. Haufroid V, Wallemacq P, VanKerckhove V, Elens L, De MM, Eddour DC *et al.* CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. *Am J Transplant* 2006; 6(11):2706-2713.
21. Lamba J, Lamba V, Schuetz E. Genetic variants of PXR (NR1I2) and CAR (NR1I3) and their implications in drug metabolism and pharmacogenetics. *Curr Drug Metab* 2005; 6(4):369-383.
22. Zhang J, Kuehl P, Green ED, Touchman JW, Watkins PB, Daly A *et al.* The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. *Pharmacogenetics* 2001; 11(7):555-572.
23. Tirona RG, Kim RB. Nuclear receptors and drug disposition gene regulation. *J Pharm Sci* 2005; 94(6):1169-1186.
24. MacPhee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A *et al.* The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. *Am J Transplant* 2004; 4(6):914-919.
25. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. *Clin Pharmacol Ther* 2002; 72(6):660-669.
26. Kuypers DR, Claes K, Evenepoel P, Maes B, Coosemans W, Pirenne J *et al.* Time-related clinical determinants of long-term tacrolimus pharmacokinetics in combination therapy with mycophenolic acid and corticosteroids: a prospective study in one hundred de novo renal transplant recipients. *Clin Pharmacokinet* 2004; 43(11):741-762.
27. Scholten EM, Cremers SC, Schoemaker RC, Rowshani AT, van Kan EJ, den Hartigh J *et al.* AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. *Kidney Int* 2005; 67(6):2440-2447.
28. Beal SL, Sheiner L.B., Boeckmann AJ. NONMEM Users Guide (1989-2006). 2006. Icon Development Solutions. Ref Type: Computer Program
29. Moller A, Iwasaki K, Kawamura A, Teramura Y, Shiraga T, Hata T *et al.* The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. *Drug Metab Dispos* 1999; 27(6):633-636.
30. Wahlby U, Jonsson EN, Karlsson MO. Comparison of stepwise covariate model building strategies in population pharmacokinetic-pharmacodynamic analysis. *AAPS PharmSci* 2002; 4(4):E27.
31. Bonate PL. Springer, 2006; 355-364.
32. Wahlby U, Jonsson EN, Karlsson MO. Assessment of actual significance levels for covariate effects in NONMEM. *J Pharmacokinet Pharmacodyn* 2001; 28(3):231-252.
33. Haufroid V, Mourad M, Van Kerckhove, V, Wawrzyniak J, De Meyer M, Eddour DC *et al.* The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics* 2004; 14(3):147-154.
34. Wong KM, Shek CC, Chau KF, Li CS. Abbreviated tacrolimus area-under-the-curve monitoring for renal transplant recipients. *Am J Kidney Dis* 2000; 35(4):660-666.
35. Ghoshal AK, Soldin SJ. IMx tacrolimus II assay: is it reliable at low blood concentrations? A comparison with tandem MS/MS. *Clin Biochem* 2002; 35(5):389-392.
36. Napoli KL. Is microparticle enzyme-linked immunoassay (MEIA) reliable for use in tacrolimus TDM? Comparison of MEIA to liquid chromatography with mass spectrometric detection using longitudinal trough samples from transplant recipients. *Ther Drug Monit* 2006; 28(4):491-504.
37. Moreton M, Fredericks S, McKeown DA, Shiferaw EW, MacPhee IA, Johnston A *et al.* CYP3A5 genotype does not influence the blood concentration of tacrolimus measured with the Abbott immunoassay. *Clin Chem* 2005; 51(11):2214-2215.
38. Satoh S, Kagaya H, Saito M, Inoue T, Miura M, Inoue K *et al.* Lack of tacrolimus circadian pharmacokinetics and CYP3A5 pharmacogenetics in the early and maintenance stages in Japanese renal transplant recipients. *Br J Clin Pharmacol* 2008; 66(2):207-214.
39. Tada H, Satoh S, Iinuma M, Shimoda N, Murakami M, Hayase Y *et al.* Chronopharmacokinetics of tacrolimus in

kidney transplant recipients: occurrence of acute rejection. *J Clin Pharmacol* 2003; 43(8):859-865.
40. Satoh S, Tada H, Tachiki Y, Tsuchiya N, Shimoda N, Akao T *et al.* Chrono and clinical pharmacokinetic study of tacrolimus in continuous intravenous administration. *Int J Urol* 2001; 8(7):353-358.
41. Park SI, Felipe CR, Pinheiro-Machado PG, Garcia R, Tedesco-Silva H, Jr., Medina-Pestana JO. Circadian and time-dependent variability in tacrolimus pharmacokinetics. *Fundam Clin Pharmacol* 2007; 21(2):191-197.
42. Baraldo M, Furlanut M. Chronopharmacokinetics of ciclosporin and tacrolimus. *Clin Pharmacokinet* 2006; 45(8):775-788.
43. Min DI, Chen HY, Fabrega A, Ukah FO, Wu YM, Corwin C *et al.* Circadian variation of tacrolimus disposition in liver allograft recipients. *Transplantation* 1996; 62(8):1190-1192.
44. Silva HT, Jr., Yang HC, Abouljoud M, Kuo PC, Wisemandle K, Bhattacharya P *et al.* One-year results with extended-release tacrolimus/MMF, tacrolimus/MMF and cyclosporine/MMF in de novo kidney transplant recipients. *Am J Transplant* 2007; 7(3):595-608.
45. Wente MN, Sauer P, Mehrabi A, Weitz J, Buchler MW, Schmidt J *et al.* Review of the clinical experience with a modified release form of tacrolimus [FK506E (MR4)] in transplantation. *Clin Transplant* 2006; 20 Suppl 17:80-84.
46. Ahn JE, Birnbaum AK, Brundage RC. Inherent correlation between dose and clearance in therapeutic drug monitoring settings: possible misinterpretation in population pharmacokinetic analyses. *J Pharmacokinet Pharmacodyn* 2005; 32(5-6):703-718.
47. Armendariz Y, Garcia S, Lopez RM, Pou L. Hematocrit influences immunoassay performance for the measurement of tacrolimus in whole blood. *Ther Drug Monit* 2005; 27(6):766-769.
48. Brown NW, Gonde CE, Adams JE, Tredger JM. Low hematocrit and serum albumin concentrations underlie the overestimation of tacrolimus concentrations by microparticle enzyme immunoassay versus liquid chromatography-tandem mass spectrometry. *Clin Chem* 2005; 51(3):586-592.
49. Tomita T, Homma M, Yuzawa K, Ohkohchi N, Hori T, Kaneko M *et al.* Effects of hematocrit value on microparticle enzyme immunoassay of tacrolimus concentration in therapeutic drug monitoring. *Ther Drug Monit* 2005; 27(1):94-97.
50. Lemahieu W, Maes B, Verbeke K, Rutgeerts P, Geboes K, Vanrenterghem Y. Cytochrome P450 3A4 and P-glycoprotein activity and assimilation of tacrolimus in transplant patients with persistent diarrhea. *Am J Transplant* 2005; 5(6):1383-1391.
51. Sato K, Amada N, Sato T, Miura S, Ohashi Y, Sekiguchi S *et al.* Severe elevations of FK506 blood concentration due to diarrhea in renal transplant recipients. *Clin Transplant* 2004; 18(5):585-590.