TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Kidney transplant outcomes are related to tacrolimus, mycophenolic acid and prednisolone exposure in the first week

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Keywords
immunosuppression, kidney transplantation, mycophenolic acid, prednisolone, tacrolimus.

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Conflicts of Interest
KB and CS have received a research grant from Roche Pharmaceuticals. SC has been on Advisory Boards for Janssen-Cilag, Roche, Novartis and Wyeth. DJ has received speakers' honoraria, travel sponsorships, research grants and consultancy fees from Janssen-Cilag and Roche. NI has received honoraria, travel sponsorships, research grants and consultancy fees from Amgen, Roche, Astellas, Pfizer, Merck Sharp and Dohme and Bristol Myers Squib. There are no other disclosures for any of the other authors.

Received: 4 March 2012
Revision requested: 21 April 2012
Accepted: 21 July 2012
Published online: 5 September 2012

doi:10.1111/j.1432-2277.2012.01553.x

Summary

This study analysed associations between tacrolimus, mycophenolic acid (MPA) and prednisolone exposures on day 4 and month 1 post kidney transplant and clinical outcomes. Area under the concentration-time curve (AUC) for each drug was estimated using validated multiple regression-derived limited sampling strategies. Multivariate logistic regression was used to associate drug exposure with clinical outcomes. One hundred and twenty subjects were studied. Between-subject variability in dose-adjusted exposure to each medication was high. Both day 4 tacrolimus and MPA exposures were independently predictive of delayed graft function (2.6 change in odds for a standard deviation (SD) increase in tacrolimus $AUC_{0–12}$, $P = 0.02$; 0.23 change in odds for a SD increase in MPA $AUC_{0–12}$, $P = 0.02$). Both day 4 MPA and total prednisolone exposures were independently predictive of rejection (0.20 change in odds for a SD increase in MPA $AUC_{0–12}$, $P = 0.04$; 0.40 change in odds for a SD increase in total prednisolone $AUC_{0–6}$, $P = 0.03$). Lowest tertile exposure to all three immunosuppressant medications imposed significantly higher odds of rejection [adjusted odds ratio 34.2 (95% CI 4.1, 284.4), $P = 0.001$]. This study highlights the importance of achieving early target exposure and suggests a potential role for individualized initial dosing or early therapeutic monitoring of all three immunosuppressive agents.

Introduction

Tacrolimus, mycophenolate and prednisolone are key immunosuppressant agents in kidney transplantation [1,2]. Combination therapy with these drugs is associated with low rates of acute rejection and high patient survival [3–5]. However, efficacy failure is still a major clinical problem, evidenced by higher rates of graft loss following a rejection episode within the first 6 months post-transplant, particularly if the episode is vascular or recurrent in nature [6].

Moreover, toxicities associated with these drugs reduce tolerability and significantly impact on recipient quality of life and patient and graft survival [3–5,7]. Outcomes of kidney transplantation could potentially be improved by greater dosage individualization of these medications.

Tacrolimus has a narrow therapeutic window and displays large pharmacokinetic variability [8]. Therapeutic drug monitoring (TDM) of tacrolimus is routinely performed to improve drug efficacy and safety. Currently, whole blood trough concentrations (C_0) are typically used to adjust tacrolimus dosing. However, conflicting data exist regarding the correlation of C_0 values with full dose interval area under the concentration-time curve ($AUC_{0–12}$) [9–20] and clinical outcomes [21–24], such that an expert consensus document suggested that $AUC_{0–12}$ might be the preferable measure of drug exposure [25]. An $AUC_{0–12}$ target of 150–250 μg h/l was proposed, but it was further stated that prospective studies of tacrolimus AUC–based TDM were required.

Mycophenolate mofetil (MMF) is typically administered at a fixed dose, and mycophenolic acid (MPA; the active constituent of MMF) concentrations are not routinely monitored. Reasons for this relate to the complexities of MPA pharmacokinetics, which make accurate measurement of MPA exposure difficult [26,27], and conflicting results from randomized controlled trials (RCTs) regarding the benefits of TDM-guided dosing over standardized dosing [28–30]. However, studies have shown ≥10-fold variation in dose-normalized MPA exposure [31], suggesting that adequate exposure may not be achieved in all individuals with standardized dosing. In addition, multiple studies have linked low drug concentrations with acute rejection [28–30,32–34], highlighting the clinical significance of underexposure. These data suggest that individualized dosing may be advantageous.

Prednisolone is also dosed in a standardized (milligram per kilogram) manner, and prednisolone concentrations are not measured in clinical practice. However, marked between-subject variability in total and free prednisolone pharmacokinetics has been demonstrated [35–38], and a study of 52 lung transplant recipients showed that conventional dosing overdoses the majority of recipients [36]. These data, in conjunction with the fact that prednisolone toxicities are frequently apparent and have substantial clinical impact, provide rationale for investigation of prednisolone TDM.

This study examined tacrolimus, MPA and prednisolone exposure in the first week and at month 1 post-transplant following routine dosing of these medications. In addition, it analysed for associations between exposure to these drugs and the clinical outcomes of acute rejection, delayed graft function (DGF) and new onset diabetes after transplantation (NODAT).

Methods

Participants

A prospective, observational cohort study was conducted between August 2009 and December 2011 as part of a PhD research project to investigate risk factors for BK viraemia. Adults (>18 years) undergoing living or deceased donor kidney transplant surgery at the Princess Alexandra Hospital (Brisbane, Australia) were recruited. Patients were eligible for inclusion if they were planned to receive combination treatment with tacrolimus, MMF and prednisolone post-transplant and were willing to provide informed consent. Of 174 patients approached for this study, 158 (91%) were enrolled. Of these individuals, 120 provided blood samples suitable for estimation of tacrolimus, MPA and prednisolone exposure on day 4 and at month 1 post-transplant and thus were included in this study. The Princess Alexandra Hospital and University of Queensland Ethics Committees approved the study protocol.

Immunosuppression

Immunosuppression was administered as per unit protocol. Induction therapy included 20 mg intravenous basiliximab (Simulect®: Novartis, East Hanover, NJ, USA) pre and 4 days postoperatively and 500 mg intravenous methylprednisolone (Solu Medrol®: Pfizer, NY, USA) pre and 12 h postoperatively. Tacrolimus (Prograf®: Janssen-Cilag, MacQuarie Park, Australia) was initiated preoperatively at an oral dose of 0.075 mg/kg twice daily and continued at this dose until C_0 values were measured on day 4 post-transplant. Thereafter, dose was adjusted to achieve individualized target C_0 values according to recipient immunological and toxicity risk status [generally, 6–10 μg/l over the first 3 months post-transplant]. Oral MMF (Cellcept®: Roche, Dee Why, Australia) was initiated preoperatively at 1000 mg twice daily with dose adjustment for toxicity or rejection. Oral prednisolone (Panafcortelone®: Aspen Pharmacare, St Leonards, Australia) was initiated on the first postoperative day at a dose of 0.3 mg/kg ideal body weight (IBW) once daily, with IBW estimated according to Broca's formula [39]:

$$\text{Weight (kg)} = \text{height (cm)} - 100.$$

Prednisolone was maintained at this dose until month 1 post-transplant. Thereafter, it was tapered down to 5–7 mg/daily by 6 months. Modification of immunosuppression was for efficacy, tolerability and safety and occurred at the discretion of treating physicians throughout the period of follow-up. Physicians were blinded to AUC results.

Clinical data

The following data were collected at baseline: patient age, gender, body weight, height, body mass index (BMI), cause of end-stage renal disease, duration of dialysis, dialysis modality, first versus subsequent transplant, living versus deceased donor transplantation, panel reactive antibody (PRA) level, human leukocyte antigen (HLA) mismatches and cytochrome P450 3A5 (CYP3A5) genotype. Warm and cold ischaemic times were recorded following the transplant operation.

Acute rejection episodes within the first month post-transplant were recorded. All were biopsy proven and graded according to the revised Banff '97 criteria [40]. DGF was defined by a requirement for dialysis within 72 hours of the transplant operation [41]. NODAT within 3 months of transplantation was diagnosed by a fasting plasma glucose ≥126 mg/dl (7 mmol/l) on two occasions, symptoms of diabetes plus random plasma glucose ≥200 mg/dl (11.1 mmol/l) or a 2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test [42].

Drug exposure

Drug exposure was estimated on day 4 and at month 1 post-transplant by applying concentrations measurements taken predose (C_0) and at 1 (C_1), 2 (C_2) and 4 (C_4) hours postdose in limited sampling strategy (LSS) equations. These had been previously developed and/or validated in our transplant population [35,43,44] and were chosen because of clinically acceptable predictive power (bias and imprecision estimates of <15%) and common sampling times for all three drugs. Specifically, the LSS equations were as follows:

$$\text{Tacrolimus: } AUC_{0-12} = -5.385 + 3.337C_0 + 0.96C_1 + 1.402C_2 + 6.01C_4$$

$$\text{MPA: } AUC_{0-12} = 8.55 + 5.68C_0 + 4.81C_4$$

$$\text{Total prednisolone: } AUC_{0-6} = -0.156 + 1.155C_1 + 1.565C_2 + 2.496C_4$$

$$\text{Free prednisolone: } AUC_{0-12} = 1.09 + 0.79C_1 + 1.95C_2 + 4.49C_4$$

In addition, total and free prednisolone AUC_{0-4} was estimated using the linear trapezoidal rule to allow direct comparison of total and free prednisolone exposure.

Day 4 was chosen as the first time point for measuring drug exposure because both tacrolimus and MPA would be expected to be at steady-state by day 4 (mean apparent half-lives 15.6 h and 17.9 h, respectively, in adult kidney transplant recipients [8,45]). For prednisolone, $t_{1/2}$ is short (typically 2–4 h [46]), with the consequence that drug exposure is often negligible by the end of the dosing interval and steady-state is never achieved under a typical once daily maintenance regimen used in transplant recipients (<50 mg).

Drug concentration measurements

Tacrolimus concentrations were determined in whole blood using high performance liquid chromatography (HPLC) with tandem mass spectrometric detection [47,48]. This assay was linear over the range 0.5–50 μg/l. Accuracy ranged from 101.3% to 103.4% and imprecision was less than 5%.

MPA concentrations were determined in plasma using HPLC with tandem mass spectrometric detection. This assay was based on that of Brown *et al.* [49] and was linear over the range 0.25–25 mg/l. Accuracy ranged from 99.8% to 103.7% and imprecision was <6%.

Total and free prednisolone concentrations were determined in plasma and plasma ultrafiltrate, respectively, using ultra HPLC with tandem mass spectrometric detection [50]. Plasma ultrafiltrate was prepared using temperature-controlled ultrafiltration. This assay was linear over the range 1.0–2000 nmol/l. Intra-assay coefficient of variation (CV) was <5% and interassay CV was <10%.

Pharmacogenetic analysis

CYP3A5 genotyping was performed on blood samples from each study patient. Genomic DNA was extracted from whole blood samples using a QIAamp deoxyribonucleic acid mini kit (Qiagen, Hilden, Germany) and was stored at 4 °C until analysis. Real-time polymerase chain reaction (PCR) was performed using a 7900 Real-Time PCR System (Applied Biosystems, Melbourne, Australia). PCR conditions were 10 min at 95 °C, then 50 cycles of 15 s at 92 °C and 1 min 30 s at 69 °C. CYP3A5 6986A>G (rs776746) allelic discrimination was undertaken using a Custom TaqMan®: Applied Biosystems, Melbourne, Australia. Single Nucleotide Polymorphism (SNP) Genotyping Assay (Applied Biosystems, Melbourne, Australia) and VIC and FAM reporters.

Statistical analysis

Data were assessed for normality of distribution and if non-normal, transformed where possible. Descriptive statistics used were mean ± standard deviation (SD) or median with interquartile range (IQR) for continuous variables, and percentages for categorical variables. For univariate comparisons, Chi squared test, Fisher's exact test, unpaired *t*-test, Wilcoxon rank sum test and Wilcoxon

signed-rank test were used where appropriate. The degree of association between tacrolimus C_0 and $AUC_{0–12}$ and total and free prednisolone $AUC_{0–4}$ were assessed using the Spearman's correlation coefficient test. Multivariate logistic regression with stepwise elimination was used to examine the influence of patient clinical characteristics on exposure to each of the immunosuppressant medications. Covariates examined during modelling included drug dose, immunosuppressant co-therapy exposure, patient age, gender, body weight, estimated glomerular filtration rate (eGFR) [51], haematocrit, serum albumin and total bilirubin and alanine transferase levels. In addition, the influence of CYP3A5 genotype on tacrolimus exposure was tested. For these analyses, drug exposure, the dependent variable, was divided into tertiles according to AUC values. Covariates eGFR, haematocrit and serum albumin were also converted into tertiles, whereas alanine transferase and bilirubin levels were divided into two groups according to whether values were above or below the upper limits of their normal ranges (45 IU/l and 20 μmol/l respectively). Multivariate logistic regression with stepwise elimination was also used to test the influence of drug exposure and relevant covariates on the clinical outcomes of acute rejection, DGF and NODAT. For these analyses, the influences of total and free prednisolone exposure were tested in separate models because of colinearity between the two. For the same reason, the influences of tacrolimus C_0 and $AUC_{0–12}$ were also tested in separate models. For those drugs identified using logistic regression analysis as significantly influencing clinical outcomes, receiver operating characteristic (ROC) curve analyses were performed to provide threshold data regarding AUC values that might predict risk with optimal sensitivity and specificity. The null hypothesis was rejected at the 0.05 level. All statistical analyses were performed using STATA Version 11.1, StataCorp, TX, USA.

Table 1. Summary of baseline characteristics of kidney transplant recipients.

Characteristic	All subjects
Number of patients	120
Age (years)	49 ± 13
Male *n* (%)	83 (69)
CYP3A5 6989 genotype	
AA *n* (%)	4 (3)
AG *n* (%)	13 (11)
GG *n* (%)	103 (86)
Race	
Caucasian *n* (%)	109 (91)
Asian *n* (%)	5 (4)
Other *n* (%)	6 (5)
Aetiology of kidney failure	
Glomerulonephritis *n* (%)	40 (33)
Polycystic kidney disease *n* (%)	19 (16)
Vesicoureteric reflux *n* (%)	11 (9)
Diabetes *n* (%)	5 (4)
Others *n* (%)	30 (25)
Unknown *n* (%)	15 (13)
Dialysis modality	
Haemodialysis *n* (%)	80 (67)
Peritoneal dialysis *n* (%)	25 (21)
Pre-emptive transplantation *n* (%)	15 (13)
Dialysis duration (months)	30 [13.5, 44.5]
Transplant number	
1 *n* (%)	109 (91)
2 *n* (%)	9 (7)
3 *n* (%)	2 (2)
Transplant type	
Living donor *n* (%)	42 (35)
Deceased donor *n* (%)	78 (65)
Panel reactive antibody level *n* (%)	
0%	94 (78)
1–20%	16 (13)
>20%	10 (8)
Human leukocyte antigen mismatches	4 (2, 5)

Values expressed as a number (percentage), except median [IQR] for dialysis duration and human leukocyte antigen mismatches and mean ± SD for age.

Results

Baseline characteristics of study participants are shown in Table 1. Clinical and biochemical parameters on day 4 compared with month 1 post-transplant are presented in Table 2.

Pharmacokinetic analysis

Tacrolimus

Dose-adjusted tacrolimus $AUC_{0–12}$ varied 20- and 12-fold between recipients on day 4 and at month 1, respectively, whereas dose-adjusted C_0 varied 31- and 18-fold. There was no significant difference in median (IQR) dose-adjusted tacrolimus $AUC_{0–12}$ or C_0 between the two time-points (Table 2). On day 4, tacrolimus $AUC_{0–12}$ values were below or above the proposed target range of 150–250 μg h/l [25] in 38% and 12% of recipients, respectively, whereas at month 1, values were below or above the target range in 42% and 2%. Coefficient of correlation (r) between tacrolimus C_0 and $AUC_{0–12}$ on day 4 was 0.74 ($P < 0.0001$). At month 1, coefficient of correlation (r) was 0.68 ($P < 0.0001$).

The only covariate independently predictive of higher exposure to tacrolimus on day 4 was homozygosity for the CYP3A5 6989 G allele [AOR 4.3 (95% CI 1.4, 12.9), $P = 0.009$]. No covariate tested was predictive of tacrolimus exposure at month 1.

Table 2. Clinical and biochemical parameters of kidney transplant recipients on day 4 and at month 1 post-transplantation.

Parameter	Day 4	Month 1	*P*-value
Body weight (kg)	81 [71, 95]	79 [69, 93]	<0.0001
Serum eGFR (ml/min/1.73 m^2)	19 [9, 42]	48 [40, 60]	<0.0001
Serum albumin (g/l)	29 [27,31]	38 [35,40]	<0.0001
Haematocrit	0.28 [0.25, 0.31]	0.34 [0.32, 0.37]	<0.0001
Total bilirubin (μmol/l)	11 [9, 12]	12 [10, 14]	0.06
Aspartate transaminase (IU/l)	22 [16, 29]	15 [12, 20]	<0.0001
Alanine transaminase (IU/l)	25 [17, 36]	27 [18, 40]	0.3
Tacrolimus dose (mg/day)	13 (2, 18)	10 (3, 28)	0.05
Tacrolimus $AUC_{0–12}$ (μg.h/l)	179.8 [13 0.0, 214.5]	160.7 [134.3, 191.0]	0.05
Tacrolimus $AUC_{0–12}$/dose (μg.h/lmg)	14.8 [10.6, 19.2]	16.6 [10.8, 23.2]	0.09
Tacrolimus C_0 (μg/l)	9.9 [7.9, 12.6]	9.0 [7.7, 10.4]	0.006
Tacrolimus dose C_0/dose (μg/l/mg)	0.8 [0.6, 1.3]	0.9 [0.5, 1.3]	0.4
Mycophenolate mofetil dose (mg/day)	2000 (1500, 3000)	2000 [1500, 2500]	0.16
MPA $AUC_{0–12}$ (mg h/l)	29.8 [23.6, 40.8]	33.6 [25.3, 41.9]	0.02
MPA $AUC_{0–12}$/dose (mg h/l/g)	14.9 [11.8, 20.9]	17.2 [13.5, 20.8]	0.005
Prednisolone dose (mg/day)	25 (15, 30)	20 (15, 30)	<0.0001
Total prednisolone $AUC_{0–6}$(nmol h/l)	4542 [3898, 5079]	5435 [4939, 6179]	<0.0001
Total prednisolone $AUC_{0–6}$/dose (nmol h/l/mg)	190 [156, 229]	264 [231, 312]	<0.0001
Free prednisolone $AUC_{0–12}$ (nmol h/l)	941 [807, 1126]	816 [668, 950]	<0.0001
Free prednisolone $AUC_{0–12}$/dose (nmol h/l/mg)	40 [34, 51]	40 [33, 47]	0.06

Values expressed are median [IQR] except for median (range) for drug doses.
eGFR, estimated glomerular filtration rate; MPA, mycophenolic acid.

Mycophenolic acid

Dose-adjusted MPA $AUC_{0–12}$ varied 8- and 13-fold between recipients on day 4 and at month 1 respectively. Median (IQR) dose-adjusted MPA $AUC_{0–12}$ was significantly lower on day 4 compared with at month 1 (Table 2). On day 4, MPA $AUC_{0–12}$ values were below and above the recommended target range of 30–60 mg h/l [52] in 50% and 1% of recipients, respectively, whereas at month 1, $AUC_{0–12}$ values were below and above the target range in 36% and 6%. Coefficient of correlation (r) between MPA C_0 and $AUC_{0–12}$ was 0.80 on day 4 ($P < 0.0001$) and 0.55 at month 1 ($P < 0.0001$).

Higher eGFR was the only factor independently predictive of higher exposure to MPA on day 4 [AOR 1.8 (95% CI 1.2, 2.8), $P = 0.008$]. At month 1, higher serum albumin concentration, lower body weight and lower eGFR were all independently predictive [AOR 2.0 (95% CI 1.2, 3.3), $P = 0.006$; AOR 0.97 (95% CI 0.95, 0.99), $P = 0.03$; and AOR 0.4 (95% CI 0.2, 0.6), $P < 0.0001$ respectively].

Prednisolone

Dose-adjusted total prednisolone $AUC_{0–6}$ varied 6 and 2-fold between recipients on day 4 and at month 1, respectively, whereas dose-adjusted free prednisolone $AUC_{0–12}$ varied 7- and 6-fold. Median (IQR) dose-adjusted total prednisolone $AUC_{0–6}$ was significantly lower on day 4 compared with month 1 (Table 2). Alternatively, there was no significant difference in median (IQR) dose-adjusted free prednisolone $AUC_{0–12}$ between the two time-points. Correlation (r) between total and free prednisolone $AUC_{0–4}$ was 0.73 on day 4 ($P < 0.0001$) and 0.57 ($P < 0.0001$) at month 1.

Female gender was the only covariate predictive of higher exposure to total prednisolone on day 4 [AOR 2.9 (95% CI 1.2, 6.9), $P = 0.02$]. At month 1, female gender and lower body weight were independently predictive [AOR 4.03 (95% CI 1.5, 10.9), $P = 0.006$ and AOR 0.94 (95% CI 0.92, 0.97); $P < 0.001$]. Lower eGFR was independently predictive of higher exposure to free prednisolone on day 4 [AOR 0.4 (95% CI 0.3, 0.7), $P < 0.0001$]. At month 1, lower body weight and lower eGFR were independently predictive [AOR 0.96 (95% CI 0.94, 0.99), $P = 0.002$ and AOR 0.6 (95% CI 0.3, 0.9), $P = 0.02$ respectively].

Clinical outcomes

Efficacy

The overall incidence of acute rejection within the first month post-transplant was 10% (12/120). Median (range) time to first rejection episode was 7 days [3,28]. Median (IQR) day 4 exposure to MPA and total prednisolone was significantly lower in those with versus without rejection [19.6 mg h/l (17.1, 27.1) vs. 31.1 mg h/l (24.6, 41.3), $P = 0.004$ and 3977 nmol h/l (2457, 4590) vs. 4558 nmol.h/l

Table 3. Drug exposure at day 4 and month 1 stratified according to clinical outcomes.

Outcome	*n* (%)	Tacrolimus AUC_{0-12}	P	MPA AUC_{0-12}	*P*	Total prednisolone AUC_{0-6}	*P*	Free prednisolone AUC_{0-12}	*P*
Day 4									
BPAR*	12 (10)	186 [108, 313]	0.88	19.6 [17.1, 27.1]	0.004	3977 [2457, 4590]	0.01	813 [661,1069]	0.48
No BPAR	108 (90)	179 [133, 211]		31.1 [24.6, 41.3]		4558 [4056, 5089]		948 [643, 1070]	
DGF	24 (20)	194 [180, 230]	0.03	26.5 [18.5, 33.2]	0.02	4742 [3884, 5343]	0.73	1126 [892, 1415]	0.004
No DGF	96 (80)	166 [125, 209]		31.3 [24.6, 41.7]		4525 [3977, 4997]		923 [791, 1069]	
NODAT†	16 (13)	225 [132, 247]	0.15	NA		4793 [3392, 5213]	0.81	1018 [887, 1278]	0.30
No NODAT	104 (87)	171 [130, 205]		NA		4525 [3982, 5078]		927 [803, 1095]	
Month 1									
NODAT†	16 (13)	180 [136, 210]	0.44	NA		5682 [4956, 6369]	0.83	819 [600, 913]	0.71
No NODAT	104 (87)	161 [134, 190]		NA		5421 [4939, 6131]		812 [668, 951]	

Values are expressed as median [interquartile range]. AUC, area under the concentration-time curve; BPAR, biopsy proven acute rejection; DGF, delayed graft function; MPA, mycophenolic acid; NA, not applicable; NODAT, new onset diabetes after transplantation; *P*, *P*-value;
*Rejection occurring within the first post-transplant month.
†NODAT occurring within the first 3 post-transplant months.

(4056, 5089), $P = 0.01$ respectively]. Alternatively, there was no significant difference in day 4 tacrolimus or free prednisolone exposure between rejecters and nonrejecters (Table 3).

Using multivariable logistic regression adjusting for immunosuppressant cotherapy exposure, recipient age, first versus subsequent transplant, PRA level, HLA mismatches and the presence of DGF, both day 4 MPA AUC_{0-12} and total prednisolone AUC_{0-6} were independently predictive of rejection (0.20 change in odds for a SD increase in MPA AUC_{0-12}, SD = 12.2 mg h/l, $P = 0.04$; 0.40 change in odds for a SD increase in total prednisolone AUC_{0-6}, SD = 1078 nmol.h/l, $P = 0.03$). Alternatively, day 4 tacrolimus and free prednisolone exposure had no predictive effect. Using ROC curve analyses, the optimal MPA AUC_{0-12} cut-off value for predicting acute rejection was 23.0 mg h/l (sensitivity and specificity 80% and 75% respectively; Fig. 1). For total prednisolone, 4204 nmol h/l was identified as the optimal AUC_{0-6} cut-off value (sensitivity and specificity 73% and 71% respectively).

Study participants were also divided into two groups according to whether or not they had lowest tertile exposure to all three drugs in terms of AUC measurement. A significantly higher rate of rejection in the first month was seen in those with lowest tertile exposure to tacrolimus, MPA and total prednisolone on day 4, as compared with the rate in those with middle and highest tertile exposure (50% vs. 8%; $P = 0.001$). Figure 2 depicts this difference. Using multivariable logistic regression, lowest tertile exposure to tacrolimus, MPA and total prednisolone and the presence of DGF both imposed significantly higher odds of rejection [AOR 34.2 (95% CI 4.1, 284.4), $P = 0.001$; and AOR 7.2 (95% CI 1.2, 42.8) for the two covariates respectively]. In contrast, recipient age, first versus subsequent transplant, PRA level and number of HLA mismatches had no predictive effect.

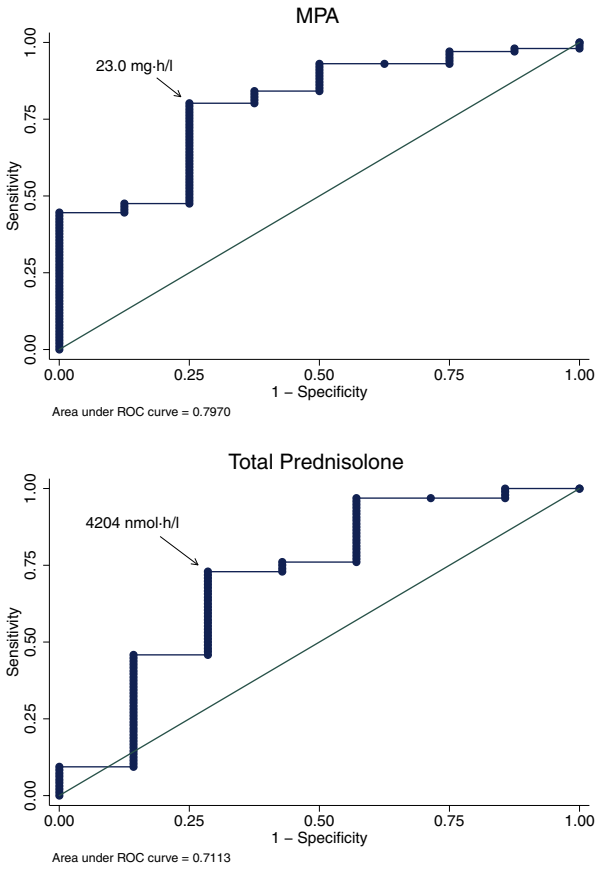

Figure 1 Receiver operating characteristic curves for the MPA and total prednisolone cut-off points for increased risk of acute rejection within the first month post-transplant.

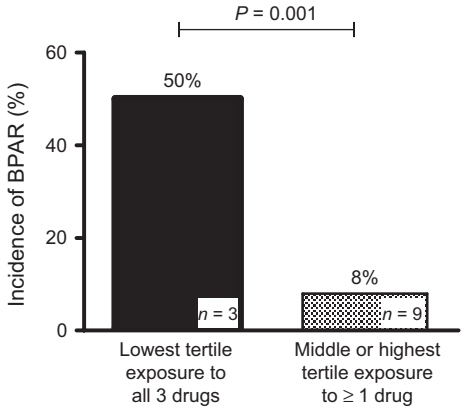

Figure 2 Incidence of biopsy proven acute rejection within the first month post-transplant in relation with drug exposure. The solid bar represents lowest tertile exposure to tacrolimus, MPA and total prednisolone, whereas the shaded bar refers to middle or highest tertile exposure to at least one drug.

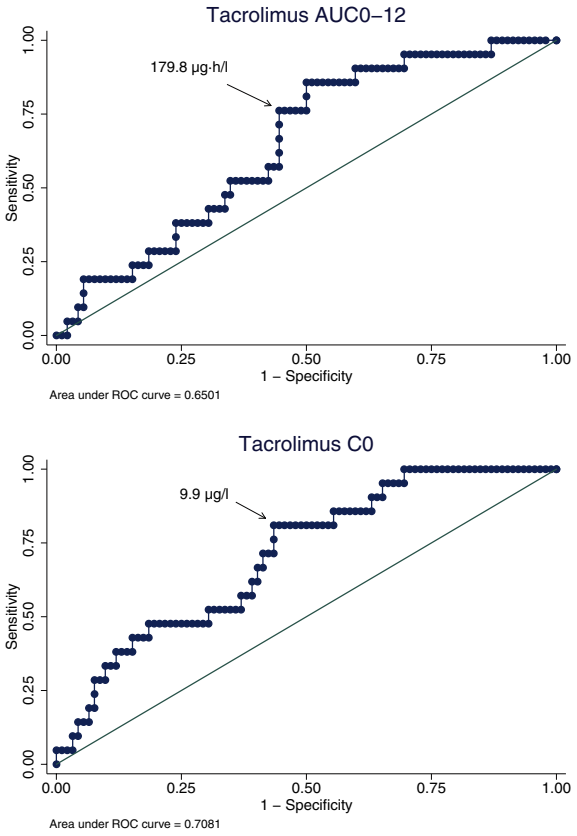

Figure 3 Receiver operating characteristic curves for the tacrolimus $AUC_{0–12}$ and C_0 cut-off points for increased risk of delayed graft function.

Toxicity

DGF was observed in 24 recipients (20%). Median (IQR) tacrolimus $AUC_{0–12}$ and C_0 and free prednisolone $AUC_{0–12}$ were significantly higher on day 4 in those with versus without DGF (Table 3). In contrast, MPA $AUC_{0–12}$ was significantly lower in those with versus without DGF, whereas total prednisolone exposure was similar between groups. Using multivariable logistic regression adjusting for donor age, warm and cold ischaemic times, living versus deceased donor transplantation and immunosuppressant cotherapy exposure, day 4 tacrolimus $AUC_{0–12}$ and C_0 and MPA $AUC_{0–12}$ were independently associated with of DGF (2.6 change in odds for a SD increase in tacrolimus $AUC_{0–12}$, SD = 53.6 µg h/l, $P = 0.02$; 2.9 change in odds for a SD increase in tacrolimus C_0, SD = 4.6 µg/l, $P = 0.006$; 0.23 change in odds for a SD increase in MPA $AUC_{0–12}$, SD = 12.5 mg·h/l, $P = 0.02$). In contrast, total and free prednisolone exposures were not significantly independently associated. Using ROC curve analyses, the optimal tacrolimus $AUC_{0–12}$ cut-off value for predicting DGF was 179.8 µg h/l (sensitivity and specificity 75% and 55% respectively; Fig. 3). The optimal tacrolimus C_0 cut-off value was 9.9 µg/l (sensitivity and specificity 81% and 57% respectively). Given that DGF likely causes alterations in MPA exposure rather than vice versa, similar analyses were not performed for MPA.

NODAT was observed in 16 recipients (13%) over the first 3 months post-transplant. No significant differences in day 4 or month 1 exposure to tacrolimus or total or free prednisolone were identified in those with versus without NODAT over this period (Table 3). Similarly, using multivariable logistic regression adjusting for patient age, BMI, cause of underlying renal disease and HLA mismatches, drug exposure on day 4 and at month 1 was not predictive of NODAT. Because of the potential confounding influence of pulse steroid therapy in those with episodes of rejection, the relationship between drug exposure and NODAT was examined separately in those with versus without rejection. In these subgroups, as in the study cohort as a whole, neither tacrolimus nor total or free prednisolone exposure was independently associated with NODAT over the time period examined.

Discussion

This study is the first to simultaneously assess exposure to tacrolimus, MPA and prednisolone and thus to examine both the individual and combined influence of these drugs on clinical outcomes post kidney transplantation. It confirms wide between-subject variability in dose-adjusted exposure to each of these commonly used immunosuppressant medications. In addition, it identifies a limited number of determinantes of drug exposure, and

demonstrates important associations between drug exposure and clinical outcomes.

Our finding of an association between homozygosity for the CYP3A5 6989G allele and higher early exposure to tacrolimus is consistent with reports of lack of CYP3A5 activity in such patients [53]. At month 1, no covariate was predictive of tacrolimus exposure, consistent with the practice of TDM and subsequent dose adjustment to achieve target exposure.

The only factor predictive of higher exposure to MPA on day 4 was a higher eGFR. This can be explained by reduced renal excretion of 7-O-MPA glucuronide (MPAG; the major MPA metabolite) and organic anions in those with worse kidney function and hence decreased availability of plasma protein sites for free MPA binding. This leads to an increase in MPA free fraction, which in turn increases MPA clearance [27]. Consistent with this notion, in the present study, lower MPA exposure was observed in the subgroup of patients with DGF. Interestingly, by month 1, we observed the opposite effect of eGFR on MPA $AUC_{0–12}$, with higher eGRF associated with lower MPA exposure. A similar duality of the effect of renal function on MPA exposure has been noted by Naesens *et al.* [54], who described a positive association between MPA $AUC_{0–12}$ and kidney function in the context of severe graft dysfunction in the early post-transplant period, compared with a negative association in stable transplant recipients (eGFR 25–80 ml/min/1.73 m^2). Postulated mechanisms for this include increased enterohepatic recirculation of MPA caused by accumulation and subsequent increased biliary excretion of MPAG in those with moderately impaired renal function, or impairment of liver clearance of MPA caused by the influence of uraemic toxins on uridine diphosphate-glucuronosyl transferase activity [54,55].

Female gender was found to be associated with higher day 4 exposure to total prednisolone, whereas at month 1, both female gender and lower body weight had independent influence. For free prednisolone, lower eGFR was the only predictive factor on day 4, whereas lower body weight was predictive at month 1. Although there has been only limited previous study of contributors to prednisolone pharmacokinetic parameters, these data are consistent with earlier reports. Female sex has been associated with lower apparent oral clearance of both total and free prednisolone in kidney transplant recipients ($n = 42$) [37,56]. Studies in both lung ($n = 52$) and kidney ($n = 42$) transplant recipients have shown significantly higher total prednisolone exposure ($AUC_{0–6}$) in females compared with males [36,37]. To date, no clear explanation has been proposed for these sex-based differences in prednisolone pharmacokinetic parameters. An impact of impaired kidney function on total and free prednisolone exposure has been demonstrated in studies comparing healthy controls with dialysis patients ($n = 7$) [57] and uraemic subjects ($n = 6$) [58] respectively. This can be explained by reduced renal excretion of prednisolone [59], although uraemia-induced reductions in hepatic clearance may also contribute [57].

No association was observed between tacrolimus exposure ($AUC_{0–12}$ or C_0) and acute rejection. This is possibly reflective of adequate to high tacrolimus exposure in our cohort [median (IQR) tacrolimus C_0 9.9 μg/l (7.9, 12.6) on day 4 and 9.0 μg/l (7.7, 10.4) at month 1]. Interestingly, despite tacrolimus C_0 values suggestive of adequate to high exposure, a substantial proportion of study participants (38% on day 4 and 42% at month 1) had $AUC_{0–12}$ values below the lower limit of the proposed target range (150–250 μg h/l) [25]. This may possibly reflect the poor-to-moderate correlation observed between $AUC_{0–12}$ and C_0, or alternatively, inappropriateness of the nonvalidated $AUC_{0–12}$ target range. Of note, the consensus guidelines that suggested this target range made no mention of induction or cotherapy use [25]. Similarly, some of the original studies on which this target range was based on either failed to report immunosuppressant cotherapy use [60] or involved patients on variable cotherapy [22].

We did observe an association between higher tacrolimus exposure ($AUC_{0–12}$ and C_0) on day 4 and the presence of DGF. Kuypers *et al.* [61] reported a similar finding, describing higher C_0 values in those with DGF up until day 4 post-transplant. As discussed by Kuypers *et al.* [61], this may simply be indicative of the nephrotoxic properties of tacrolimus, or instead be attributable to the influence of renal insufficiency on tacrolimus disposition (possibly mediated by uraemia-induced reductions in CYP3A4/3A5 and P-glycoprotein activity) [62,63]. Interestingly, in Kuyper's study, the difference in exposure was only observed in those with CYP3A5 GG genotype (CYP3A5 'nonexpressers'). We were unable to demonstrate a similar significant impact of genotype on exposure in the subgroup with DGF (data not shown), likely influenced by the fact that the vast majority of individuals in our study with DGF (21/24) were of the GG genotype.

Our finding of an association between low early exposure to MPA and acute rejection is also consistent with the findings of previous investigations [29,30]. Of significance, we found that with conventional 'fixed' dosing of MMF, MPA exposure was below the lower limit of the recommended target range [52] in 51% of recipients on day 4 and 36% at month 1. Similarly, high numbers with subtherapeutic exposure have been seen in other trials [28,30,34,64], although most commonly in the context of ciclosporin cotherapy (known to inhibit enterohepatic recycling of MPA). In response to this, two recently

published RCTs (in ciclosporin and tacrolimus cotreated kidney transplant recipients respectively) investigated early intensified compared with standard dosing of mycophenolate [34,64]. In both trials, significantly higher MPA exposure was seen in the first week with intensified dosing. In the first trial, acute rejection was also significantly less common in the intensified group (13.8% vs. 19.3%, $P = 0.03$) [64], whereas in the second trial, a strong trend to fewer rejection episodes was observed (11.8% vs. 28.4%, $P = 0.05$) [34]. It should be noted, however, that this approach increases the risk of overexposure in some recipients. An alternative approach is to perform TDM as early in the first week as is practical, taking into account the steady-state period of MPA, and then to augment dosing in those below target. Comparative efficacy and toxicity associated with these two approaches requires testing in a randomized controlled trial setting.

For the first time, a relationship between total prednisolone exposure and rejection was seen in this study. Only very minimal data exist regarding the association of prednisolone pharmacokinetic parameters with clinical outcomes. A study of 35 kidney transplant recipients treated with prednisolone and azathioprine showed that rejection more frequently resulted in graft loss in those with high compared with low total prednisolone clearance (80% vs. 21%; $P < 0.05$) [38]. A study of 62 kidney transplant recipients found that $AUC_{0–4}$ was 15% higher for free prednisolone and 20% higher for total prednisolone in those with versus without Cushingoid features [65]. In a study of 108 nondiabetic kidney transplant recipients, free prednisolone $AUC_{0–12}$ was independently predictive of postchallenge hyperglycaemia [66].

Interestingly, we failed to demonstrate a relationship between free prednisolone and rejection. Our decision to measure free in addition to total prednisolone concentrations was based on the fact that prednisolone displays nonlinear binding to the plasma proteins albumin and transcortin, with a free fraction ranging from <0.1 to 0.5 [67]. Subsequently, as demonstrated by our data, total and free prednisolone concentrations may not always be highly correlated. Given that the biological effects of prednisolone are caused by unbound drug binding at receptor sites, free drug measurement, rather than total, would be expected to be a better predictor of efficacy and toxicity. In our cohort, however, although free prednisolone $AUC_{0–12}$ was numerically lower in those with rejection, the difference failed to reach statistical significance. Reasons for this may include a type 2 statistical error, or failure of the chosen LSS to adequately capture free prednisolone exposure.

Because of the potential adverse impact of corticosteroid-related toxicities on kidney transplant outcomes, corticosteroid minimization or avoidance is a major desire of both patients and their transplant physicians. However, because of higher rates of acute rejection and chronic allograft nephropathy [68], widespread application of such protocols has been limited. Our data suggest that TDM of prednisolone might assist with better delineation of the balance between efficacy and toxicity and potentially allow for safer reduction of exposure. Further examination of the relationship between total and free prednisolone plasma concentrations and drug efficacy and toxicity is warranted, as are trials aimed at establishing an appropriate target range for prednisolone exposure.

Given that the combined immunosuppressive effects of all three drugs contribute to the prevention of rejection, we also defined two patient cohorts according to whether or not individuals were underexposed to all three drugs. A strikingly higher rate of rejection was observed in those with versus without lowest tertile tacrolimus, MPA and total prednisolone AUC values (50% vs. 8%, $P = 0.001$), suggesting additive impact of underexposure to each immunosuppressive medication on rejection. A similar effect was observed by Kuypers *et al.* [69], who reported a trend ($P = 0.07$) towards a higher incidence of acute rejection in kidney transplant recipients who did not simultaneously have a target tacrolimus $AUC_{0–12}$ of 150 μg h/l and a MPA $AUC_{0–12}$ of 45 mg h/l by day 7, as compared with patients who reached both target values (26.3% vs. 7.7%). Of significance, in this study, no clinical covariate was predictive of underexposure to all three drugs (data not shown). This suggests a potential role for early TDM to identify this extremely high-risk subgroup, or alternatively intensified early dosing of MMF or prednisolone.

A major limitation of this study was that sample size was based on convenience rather than power calculations. Thus, it is possible that some negative study results may be attributable to insufficient power to detect a difference rather than a lack of effect. It should be noted, however, that power calculations were problematic because of the marked paucity of data in the literature relating prednisolone (and to a lesser extent, tacrolimus) AUC monitoring to clinical outcomes. An advantage of this study is that it provides data that may enable properly powered studies to be designed in the future. A further limitation of this study relates to the fact that oral glucose tolerance testing was performed in a minority of study participants, with most cases of NODAT diagnosed via measurement of an abnormal fasting plasma glucose level. Given that fasting plasma glucose measurements are known to be insensitive at detecting NODAT (70), we may have failed to adequately characterize this outcome. Finally, we examined the association between drug exposure on day 30 and clinical outcomes in the first 3 months without taking into consideration dosage changes over this period. This

raises the possibility that our AUC estimates may not have been fully reflective of drug exposure over the entire period studied.

Irrespective, this study clearly demonstrates high variability in exposure to the three most commonly used immunosuppressant agents in kidney transplant recipients, suggesting that individualized dosing may be beneficial. Our finding of higher tacrolimus exposure in those with DGF raises the possibility that modified initial tacrolimus dosing may be warranted in those thought to be at highest risk. The associations between early exposure to MPA and total prednisolone and acute rejection highlight the importance of rapidly achieving adequate concentrations of these drugs, whereas the markedly higher rejection rate in those with low exposure to all three drugs emphasizes the protective effect of combined exposure. A potential role for intensified initial dosing may exist, or alternatively for early TDM with subsequent dose modification. For MPA, evidence exists to support this practice. For prednisolone, prospective randomized studies are required to see whether such actions translate into improved clinical outcomes.

Authorship

KB: participated in study design, performed research/study, collected data, analyzed data and wrote the paper. CS: participated in study design and revised the manuscript. DJ: participated in study design and revised the manuscript. KL: analyzed study samples and revised the manuscript. BM: analyzed study samples and revised the manuscript. JU: analyzed study samples and revised the manuscript. CH: assisted with statistical analyses and revised the manuscript. SC: participated in study design and revised the manuscript. DL: performed research/study and collected data. NI: participated in study design and revised the manuscript.

Funding

The authors have declared no funding.

Acknowledgements

K. Barraclough is currently supported by a National Health and Medical Research Council (NHMRC) Medical/Dental Post-graduate Research Scholarship. C. Staatz is currently supported by a Lions Medical Research Fellowship. D. Johnson is currently supported by a Queensland Government Health Research Fellowship. This research is supported by a NHMRC Project Grant, number 511109, and an Amgen-Transplantation Society of Australia and New Zealand Research Grant.

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