BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2008.03251.x

A limited sampling strategy for tacrolimus in renal transplant patients

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Keywords
jackknife, limited sampling strategy, tacrolimus

Received
12 February 2008
Accepted
8 June 2008
Published *OnlineEarly*
25 July 2008

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Tacrolimus trough concentration is being currently used for dose individualization.
- Limited sampling strategies (LSS) have been developed and validated for renal transplant patients.
- Earlier literature has suggested that measurement of tacrolimus AUC is more reliable than trough with respect to both rejection and nephrotoxicity.

WHAT THIS STUDY ADDS

- Four thousand renal transplants take place annually in India, with many patients prescribed tacrolimus in combination with mycophenolate and steroid.
- In this study a LSS with two points, i.e. trough and 1.5 h postdose was developed and validated to estimate AUC_{0-12}.
- The added benefit of only a single additional sample with completion of blood collection in 1.5 h and minimum additional cost makes this a viable LSS algorithm in renal transplant patients.
- In patients having tacrolimus trough concentrations outside the recommended range (<3 and >10 ng ml^{-1} in the treatment protocol in our institution) or having side-effects in spite of trough concentrations in the desired range, we can estimate AUC using this LSS for a better prediction of exposure.

AIMS

To develop and validate limited sampling strategy (LSS) equations to estimate area under the curve (AUC_{0-12}) in renal transplant patients.

METHODS

Twenty-nine renal transplant patients (3–6 months post transplant) who were at steady state with respect to tacrolimus kinetics were included in this study. The blood samples starting with the predose (trough) and collected at fixed time points for 12 h were analysed by microparticle enzyme immunoassay. Linear regression analysis estimated the correlations of tacrolimus concentrations at different sampling time points with the total measured AUC_{0-12}. By applying multiple stepwise linear regression analysis, LSS equations with acceptable correlation coefficients (R^2), bias and precision were identified. The predictive performance of these models was validated by the jackknife technique.

RESULTS

Three models were identified, all with $R^2 \geq 0.907$. Two point models included one with trough (C_0) and 1.5 h postdose ($C_{1.5}$), another with trough and 4 h postdose. Increasing the number of sampling time points to more than two increased R^2 marginally (0.951 to 0.990). After jackknife validation, the two sampling time point (trough and 1.5 h postdose) model accurately predicted AUC_{0-12}. Regression coefficient $R^2 = 0.951$, intraclass correlation = 0.976, bias [95% confidence interval (CI)] 0.53% (−2.63, 3.69) and precision (95% CI) 6.35% (4.36, 8.35).

CONCLUSION

The two-point LSS equation [$AUC_{0-12} = 19.16 + (6.75.C_0) + (3.33.C1.5)$] can be used as a predictable and accurate measure of AUC_{0-12} in stable renal transplant patients prescribed prednisolone and mycophenolate.

Introduction

Tacrolimus (Pangraf®; Panacea Biotech Ltd., New Delhi, India) is a calcineurin inhibitor widely used in renal transplant patients [1]. With improved graft survival and reduced rejection [2], it is gradually replacing ciclosporin as the primary calcineurin inhibitor.

Most renal transplant patients in this centre are prescribed an immunosuppressive regimen that includes prednisolone, mycophenolate and tacrolimus. In our post renal transplant protocol, mycophenolate dosage is based on monitoring area under the curve (AUC_{0-12}) and maintaining the therapeutic range within 30–60 mg h l^{-1} [3]. The target trough concentration of tacrolimus for the first 3 months after transplant is maintained at 5–10 ng ml^{-1} [4] and thereafter at 3–5 ng ml^{-1} [5]. Tacrolimus is started at a dose of 0.1 mg kg^{-1}, and any alteration in dose thereafter is based on monitoring trough concentration.

For tacrolimus, a drug with a narrow therapeutic index, trough measurements are currently used for dose individualization [6]. However, the possibility exists of nephrotoxicity when dosing is based on tacrolimus trough measurement alone [7]. AUC is recognized as a measure for drug exposure, and Undre *et al*. [8] have reported that patients experiencing rejection had lower AUCs than rejection-free patients. At present the literature is conflicting, with some authors reporting a poor correlation between trough concentration and AUC_{0-12} [9–11] and others reporting a good correlation on day 14 after transplant [7].

Tacrolimus trough monitoring, as a guide for dose individualization, using the microparticle enzyme immunoassay (MEIA) (IMx) method has been performed for renal transplant recipients in our centre since 2006. A retrospective analysis of that year's data (555 specimens) showed the mean trough concentration of 6.53 ng ml^{-1} (range 0.3–27) at a mean dose of 0.04 mg kg^{-1} (range 0.01–0.09). Dose individualization, if based only on trough measurements, is challenging when patients have concentrations outside therapeutically acceptable values (<3 ng ml^{-1} and >10 ng ml^{-1}, our institutional protocol) and no significant change is observed despite alterations of dose. Measurement of total exposure, as AUC, for such patients can help with dose individualization.

In addition, some of our patients within the target therapeutic range for trough concentrations have been observed to have minor side-effects. Jorgenson *et al*. [7] have shown that in spite of trough levels within the recommended ranges, patients who developed nephrotoxicity had high AUC values. In both these scenarios, estimation of total exposure of the drug as AUC would be of value.

However, a full AUC involves a minimum of 10 specimens over 12 h and is not a viable option due to practical and cost issues. Therefore, in this study we have developed and validated a cost-effective method of predicting tacrolimus AUC_{0-12} using a limited sampling strategy (LSS) equation. A comparison with a published LSS for tacrolimus in post renal transplant recipients is also reported [9].

Methods

Twenty-nine stable adult renal transplant recipients were recruited after having obtained written informed consent. Patient characteristics are listed in Table 1. The study protocol was approved by the Institutional Review Board. Patients who were stable after transplant (3–6 months post transplant), at steady-state with respect to tacrolimus kinetics and prescribed both prednisolone (10–12.5 mg day^{-1}) and mycophenolate (mofetil or enteric-coated mycophenolate sodium) as co-immunosuppressants were included in the study. The dose of tacrolimus was not fixed, since monitoring performed in the immediate post-transplant period resulted in doses being adjusted in accordance with tacrolimus trough measurements, biochemistry parameters and clinical outcome of the patient.

Common additional medication included hypolipidaemic agents, bactrim, vitamin D/calcium and antihypertensives; only one patient was on nifedipine. Five patients were on antidiabetic medication.

Two patients developed rejection within 2 weeks of transplant and were effectively treated. It is interesting that the tacrolimus trough concentration at this point was within the therapeutic range. Within the first 3 months after transplant two patients were treated for urinary tract infection, three for herpes and one for proteinuria, and the tacrolimus trough was <10 ng ml^{-1} at the time of the above incidents. However, none of these conditions was present

Table 1

Demographic characteristics of patient population

Age (years)	32 ± 10.5 (18–57)
Weight (kg)	57 ± 8 (45–73)
Time post transplant (months)	3.7 ± 0.8 (3–6)
Haematocrit (%)	39.0 ± 5.9 (31–53)
Serum urea (mg dl^{-1})	32.7 ± 10.6 (19–65)
Serum creatinine (mg dl^{-1})	1.3 ± 0.32 (0.8–2.0)
Dose of tacrolimus (mg day^{-1})	3.8 ± 1.3 (2–6)
Mycophenolate mofetil daily dose (n = 19)*, mg	1500 (1000–2500)
Mycophenolate sodium daily dose (n = 10)*, mg	1350 (540–2160)
MPA AUC (nearest test result), mg h l^{-1}	57 ± 27 (27–175)
Early rejection (within 2 weeks of transplant)†	2
Diabetes‡	5
Proteinuria (>1 g day^{-1})†	1
Treatment for urinary tract infection†	2
Treatment for herpes zoster/labialis†	3

Values expressed as mean ± standard deviation (range). *Median (range). †Number of patients treated prior to the study. ‡Number of patients with diabetes at the time of the study.

during the course of the study. Five patients were diabetic at the time of the study, but no gastric autonomic neuropathy was reported.

On the day of the study following an overnight fast, patients reported to the Clinical Pharmacology Unit at 08.00 h and a cannula was inserted into a forearm vein. Three millilitres of blood was withdrawn predose, and then at 0.5, 1, 1.5, 2, 2.5, 4, 6, 8 and 12 h postdose. Patients were allowed breakfast 2 h after medication. Kimikawa *et al.* [12] have reported that the tacrolimus concentration–time profile was influenced by postprandial administration. To maintain a standard protocol on the day of the test, patients having tacrolimus and/or mycophenolate AUC measured were allowed food only after 2 h postdose. This is similar to the study by MacPhee and colleagues [13]. However, on days other than the test day, patients were instructed to take both immunosuppressants prior to a meal (up to 1 h) and to separate them by at least half an hour.

All specimens were collected into K_3 ethylenediamine tetraaceticacid-containing vacutainer tubes, stored in a refrigerator and assayed once within 48 h. Whole blood tacrolimus concentrations were determined by the MEIA on the Abbott IMx autoanalyser (Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's instructions [14]. The interday coefficients of variation for tacrolimus were 3.2, 6.5 and 4.8% for mean concentrations of 5.0, 11 and 22 ng ml^{-1}, respectively.

Pharmacokinetic and statistical analysis

Twelve-hour pharmacokinetic profiles were obtained for 29 patients. The total measured AUC_{0-12} was calculated by the trapezoidal rule. Tacrolimus concentrations at each sampling time were correlated by linear regression analysis with the total measured tacrolimus AUC_{0-12} in all 29 patients. Those concentrations at sampling time points that showed the best correlations were combined by multiple stepwise linear regression analysis to give improved correlations with total measured AUC_{0-12}. The analysis yielded equations in the form of $AUC_{0-12} = A + A_0 \times C_0 + A_1 \times C_1 \ldots An \times Cn$, where A, A_0, An are fitted constants associated with each timed concentration, C_0, $C_1 \ldots Cn$ are concentrations at 0, 1, ... nth h postdose [15]. Prediction bias of these LSS-derived estimates was assessed by calculating the percentage of prediction error (PE%) from the formula

$$PE\% = 100\% \times (\text{LSS AUC} - \text{Total measured AUC}) / \text{Total measured AUC}$$

Prediction precision was assessed by calculating the percentage of absolute prediction error (APE%) as follows

$$APE\% = 100\% \times |(\text{LSS AUC} - \text{Total measured AUC})| / \text{Total measured AUC} \quad [11]$$

An absolute prediction error of <15% was considered clinically acceptable [11].

Jackknife validation of the limited sampling strategy equations was performed using SAS 9.1 (SAS Inc., Cary, NC, USA) [16]. For jackknife validation, the regression equation for the prediction of AUC_{0-12} was derived in a subset of patients (28 patients) including concentrations at *n* fixed time points. This sampling technique of discarding one patient at a time and fitting a new model for the remaining patients (N-1), including *n* fixed time points, was repeated. The AUC value for each patient was estimated using the jackknife regression equation. The AUC predicted for each patient by the jackknife technique was compared by intraclass correlation (ICC) [17] and paired *t*-test with the LSS estimated AUC, and the bias and precision (expressed as percentage) was calculated. ICC was calculated, as when comparing the AUC from limited sampling strategy equations with the total measured AUC, the evaluations cannot be classified as independent observations because they are determined on the same patient. In addition, the LSS equation developed by Armendáriz *et al.* was validated in the above 29 patients [9].

Results

Twenty-nine pharmacokinetic profiles in 29 patients (24 male, five female) were studied. The median (range) of tacrolimus maximum concentration (C_{max}) and time of C_{max} (t_{max}) for the study was 20.1 ng ml^{-1} (9.3–30.0) and 1.0 h (0.5–2.5). The mean ± SD (range) of trough and total measured AUC_{0-12} were 5.32 ± 2.7 ng ml^{-1} (1.8–11.8) and 115.6 ± 31.5 μg h l^{-1} (64.6–176.2), respectively. The mean (range) for apparent clearance (CL/*F*), where CL is clearance and *F* is fraction of drug absorbed, was 0.31 l h^{-1} kg^{-1} (0.14–0.71). The dose-normalized trough and AUC (normalized to a dose of 0.03 mg kg^{-1}) ranged from 1.2 to 15.0 ng ml^{-1} and 42.0 to 229.0 μg h l^{-1}, respectively. There was no difference in mean apparent clearance between diabetic and nondiabetic (0.32 l h^{-1} kg^{-1}).

Nineteen patients were co-prescribed mycophenolate mofetil and 10 were on enteric-coated mycophenolate sodium. Patients on mycophenolate mofetil had a mean tacrolimus total measured AUC_{0-12} (SD) of 114. 2 μg h l^{-1} (34.4), and those on mycophenolate sodium had an AUC (SD) of 118.1 μg h l^{-1} (26.6). Of the 29 patients, 14 had a trough concentration <5 ng ml^{-1} and 15 >5 ng ml^{-1}. The mean AUC (SD) in those with a trough <5 ng ml^{-1} was 91.6 μg h l^{-1} (17.7), whereas those with a trough >5 ng ml^{-1} had a mean AUC (SD) of 138 μg h l^{-1} (24.0). Of the 15, all except one had an $AUC_{0-12} > 118$ μg h^{-1} l^{-1}. One patient with a trough >5.0 ng ml^{-1} had an AUC of 86 μg h l^{-1}. The mean concentration time profiles of 29 patients, with standard deviation, is shown in Figure 1.

Using linear regression analysis, the best correlations between AUC and concentrations at various time points

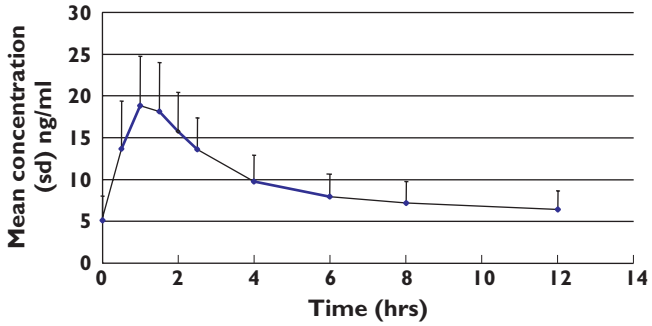

Figure 1
Mean concentration (SD) *vs.* time profiles of tacrolimus in 29 patients

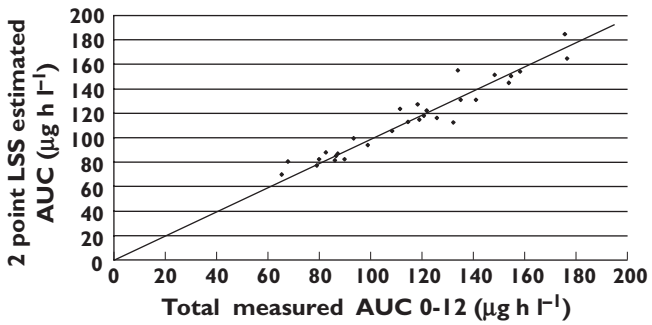

Figure 2
Regression plot of total AUC_{0-12} and two-point limited sampling strategy (LSS) AUC (Tr and I.5 h).

was at C_4 ($R^2 = 0.878$) and C_6 ($R^2 = 0.870$). The correlation between tacrolimus trough (C_0) and AUC_{0-12} was 0.582 (R^2). AUC derived from the equation using only the trough concentration had a bias [95% confidence interval (CI)] of 3.46% (–3.89, 10.81) and a precision (95% CI) of 15.70% (11.35, 20.04) in comparison with the total measured AUC_{0-12}. On forward stepwise regression, three LSS equations gave acceptable R^2 values. One equation included three time points at C_0, $C_{1.5}$ and C_4 and gave an R^2 of 0.966. Two-point LSS equations included one with C_0 and $C_{1.5}$, shown in Figure 2 ($R^2 = 0.929$) and a second with C_0 and C_4 ($R^2 = 0.907$), compared with the total measured AUC. The mean bias for the three equations ranged from 0.4 to 0.81% and the mean precision ranged from 4.61 to 6.49%. Therefore, these three equations were selected for validation via the jackknife method.

Following validation, the results for R^2, ICC and *t*-test relating to the predictive performance of each of the LSS equations are given in Table 2 and the bias and precision (as percentages) are shown in Table 3. On validation, the two-point LSS equation using only trough and 1.5-h concentrations showed good correlation with the total measured AUC, $R^2 = 0.951$ (Figure 2), ICC of 0.976 and *P*-value (paired *t*-test) of 0.965. Bland–Altman plot [18], Figure 3, shows adequate agreement and minimum bias between

Table 2
Statistical values of R^2, intraclass correlation and *t*-test (*P*-value) in the validation of the limited sampling strategy (LSS) equations

LSS equation for AUC	R^2	ICC	*t*-test (*P*-value)
14.73 + (4.38.C_0) + (2.09.$C_{1.5}$) + (4.06.C_4)	0.990	0.995	0.954
19.16 + (6.75.C_0) + (3.33.$C_{1.5}$)	0.951	0.976	0.965
23.90 + (2.74.C_0) + (7.88.C_4)	0.931	0.965	0.993

Table 3
Predictive performance of the limited sampling strategy (LSS) equations during validation

LSS equation sampling times	Mean predicted AUC, μg h l^{-1} ± SD (range)	Bias (95% CI) %	Precision (95% CI)%
C_0, $C_{1.5}$, C_4	115.7 ± 31.0 (68–181)	0.35 (–1.83, 2.53)	4.66 (3.43, 5.89)
C_0, $C_{1.5}$	115.5 ± 30.3 (70–185)	0.53 (–2.63, 3.69)	6.35 (4.36, 8.35)
C_0, C_4	115.6 ± 30.0 (72–189)	–0.01 (–3.01, 2.99)	6.52 (4.89, 8.14)

Mean total measured AUC ± SD (range) = 115.6 ± 31.5 μg h l^{-1} (64.6–176.2).

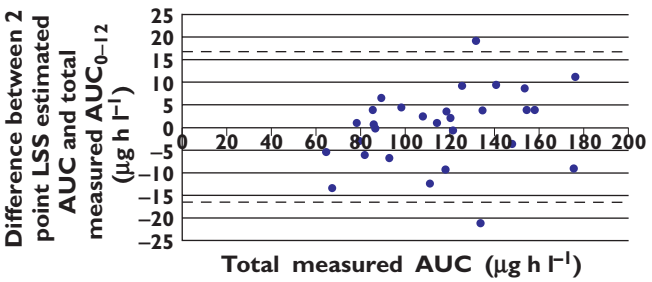

Figure 3
Bland–Altman plot for the agreement between total measured. AUC_{0-12} and two-point limited sampling strategy (LSS)-estimated tacrolimus AUC. The line represents the mean bias and the dotted lines are ± 2 times the standard deviation of the mean bias

this two-point LSS predicted AUC and total measured AUC. The power of the study, which was calculated retrospectively, was 95%.

Upon applying the LSS reported by Armendáriz *et al.* [9] to our data, the results were a mean ± SD (range) AUC of 117 ± 31.0 μg h l^{-1} (65–180), a bias (95% CI) of 1.81% (–0.90, 4.52) and a precision (95% CI) of 5.565% (3.78, 7.35) when compared with the total measured AUC.

Discussion

Tacrolimus is known to improve rejection and survival in renal transplant patients in comparison with ciclosporin

[19, 20]. Therapeutic drug monitoring (TDM) of tacrolimus is routinely undertaken to optimize therapeutic outcome [6], and trough concentration is presently a widely used parameter [6]. However, it has been reported that tacrolimus trough concentration does not correlate with efficacy of treatment. [21].

Kuypers *et al*. demonstrated that a target $AUC_{0-12} > 150$ μg h l^{-1} for tacrolimus and 45 mg h^{-1} l^{-1} for mycophenolate by day 7 post renal transplant had a role in decreasing rejection [22]. Wong *et al*. [11] have reported mean ± SD (range) AUC_{0-12} and trough concentration in an Oriental renal transplant population of 125 ± 24 μg h l^{-1} (87.7–181.9) and 6 ± 1.3 ng ml^{-1}, respectively, which was not significantly different from our data. Likewise, there was no significant difference in the apparent clearance in our data compared with the mean (range) apparent clearance 0.34 l h^{-1} kg^{-1} (0.20–0.47) reported by Mendonza *et al*. [23]. Variability of 55% in apparent clearance and 47.3% in dose-corrected AUC_{0-12} in our data highlights the variable pharmacokinetic characteristics of this population.

The cost of the immunoassay kit prohibits the routine use of 12-h monitoring, but the use of limited sampling strategy models for predicting tacrolimus AUC is feasible. The two-point LSS AUC (trough and 1.5 h) showed that 27 of 29 patients had within 85% agreement compared with the total measured AUC_{0-12}. Only one patient had a difference of 20% between LSS estimated and total measured AUC. Increasing the number of sampling time points to more than two increased R^2 marginally but added little to the bias or the precision of the LSS-estimated AUC. Stolk [10] has reported an improvement in predictive power with a combination of two samples, i.e. trough and a second sample at 1.5 h in comparison with only a single trough sample. The LSS AUC developed by Armendáriz *et al*. [9] had an acceptable bias and precision in our population. However, the two-point limited sampling strategy developed in this study has several advantages. Only one extra specimen cost is incurred, and the increased laboratory turnover time for the test is minimized. The patient can complete the test within 1.5 h, whereas all other equations in this study, including that developed by Armendáriz *et al*., involved blood collection for at least 4 h.

In clinical practice, 37% of tacrolimus trough levels measured in a year in this centre required dose modification. About a third of such modifications were estimated to be influenced differently because of an AUC rather than a trough. This proportion is likely to be different if LSS is performed in patients with unexpected troughs for the doses they receive. With implementation of LSS AUC for tacrolimus, we expect a 15% reduction in tacrolimus toxicity and 30% decline in rejections related to low tacrolimus exposure with TDM based on only trough measurement, given that our doses are lower than in most centres. Each such episode requires admission for evaluation with a kidney biopsy costing an equivalent of £102. More importantly, these episodes impact on long-term graft survival, which equates with patient survival.

To conclude, using the LSS model, including trough and 1.5-h postdose sample, provides a reliable and simple method to estimate exposure by AUC in renal transplant patients. Further work is required to determine the usefulness of this LSS equation in patients in whom it is difficult to optimize the tacrolimus dose using only trough concentration.

This study was supported by Panacea Biotech and the Fluid Grant Research Committee of Christian Medical College, Vellore. We would like to thank Mrs Catherine Saseela for her excellent work in the laboratory throughout the entire period of this study, and Mrs. Lilly Abraham for assistance in blood sample collection.

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