Low tacrolimus concentrations and increased risk of early acute rejection in adult renal transplantation

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Abstract

Background. A retrospective analysis was performed on adult renal transplant recipients to evaluate the relationship between tacrolimus trough concentrations and the development of rejection in the first month after transplant.

Methods. A total of 349 concentrations from 29 patients, measured by enzyme-linked immunosorbent assay (ELISA), were recorded. Based on an increased serum creatinine, 12 patients were considered to have organ rejection. Rejection was confirmed by biopsy in five of these. The median trough concentration of tacrolimus over the first month of therapy, or until the time of first rejection was compared in rejecters vs non-rejecters.

Results. Median trough concentrations of tacrolimus were found to be lower in biopsy-proven rejecters vs non-rejecters ($P = 0.03$) and all rejecters vs non-rejecters ($P = 0.04$). The average median concentration ($\pm$ SD) in the biopsy-proven rejecter group was $5.09 \pm 1.16 \text{ ng/ml}$, compared to $9.20 \pm 3.52 \text{ ng/ml}$ in the non-rejecter group. After exclusion of an outlier, the average median concentration in all rejecters was $5.57 \pm 1.47 \text{ ng/ml}$, compared with $9.20 \pm 3.52 \text{ ng/ml}$ in non-rejecters. A rejection rate of 55% was found for patients with a median trough concentration between 0 and 10 ng/ml. This compared with no observed rejection in patients with a median concentration between 10 and 15 ng/ml.

Conclusion. A significant relationship exists between organ rejection and median tacrolimus trough concentrations in the first month post-transplant, with patients displaying low concentrations more likely to reject. In order to minimize rejection in the first month after renal transplantation, trough concentrations greater than 10 ng/ml must be achieved.

Keywords: adult renal transplant; concentration; biopsy-proven rejection; pharmacodynamics; tacrolimus; therapeutic drug monitoring

Introduction

Tacrolimus (FK506, Prograf\textsuperscript{\textregistered}) is an immunosuppressant drug that has emerged as a valuable therapeutic alternative to cyclosporin-A for the prevention of graft rejection following kidney transplantation [1–4]. Tacrolimus whole-blood trough concentrations have been found to correlate well with area under the concentration–time curve measurements in liver, kidney, and bone-marrow transplant recipients ($r = 0.91–0.99$) [5,6]. Thus trough concentrations are a good index of overall drug exposure, and are currently used for routine monitoring as part of patient care post-transplantation. Although the therapeutic window for tacrolimus needs to be more clearly defined, a survey of transplant centres in 1997 reported that for renal transplant patients it is in the 5–20 ng/ml range [7]. At present The Queen Elizabeth Hospital Renal Unit (Adelaide, Australia) targets blood concentrations between 10 and 20 ng/ml in the first 3 months post-transplant and then between 2 and 15 ng/ml thereafter.

While a number of studies have been performed to evaluate the pharmacodynamics of tacrolimus [8–18], a greater understanding of the relationship between trough concentration measurements and the development of organ rejection is required. It is still not definitively known whether low tacrolimus trough concentrations are related to the development of organ rejection in kidney-transplant recipients, or how low trough concentrations can be before rejection is likely, with conflicting reports from different investigators [11,12,14,18]. Should the lower limit of the concentration range be 2 ng/ml, 10 ng/ml, or 20 ng/ml in the important initial time after transplantation?

The aim of this study was to retrospectively evaluate the relationship between median tacrolimus trough
concentrations and the development of organ rejection in the first month following renal transplantation.

Subjects and methods

Patients and study design

A retrospective analysis of data from 30 adult kidney-transplant recipients at a single centre was performed. Patients were receiving tacrolimus as primary immunosuppressant therapy and undergoing routine therapeutic drug monitoring. Patients underwent transplant surgery between November 1994 and December 1995, and constituted the ‘tacrolimus arm’ of participants in a hospital clinical trial comparing tacrolimus to cyclosporin-A therapy. Permission to collect and analyse data, available as part of the clinical trial, were obtained from the pharmaceutical company (Janssen Cilag, letter dated 27 March 1996). The clinical trial had been approved by The Queen Elizabeth Hospital (Adelaide, Australia) Medical Research Ethics Committee.

Immunosuppressive therapy

During the study period, all patients received a triple immunosuppressive regimen of tacrolimus, prednisolone, and azathioprine. The recommended initial dose of tacrolimus was 0.075 mg/kg, twice daily. Tacrolimus was administered orally or via a nasogastric tube, beginning on the day of transplantation. Subsequent doses were adjusted on the basis of clinical evidence of efficacy and toxicity, and to try and maintain target whole-blood trough concentrations within the range 10–20 ng/ml within the first 3 months after transplant. All patients received 1 g of intravenous methylprednisolone on the day of transplantation and 500 mg intravenously the following day. Oral maintenance prednisolone therapy was subsequently initiated at a dose of 30 mg per day, reduced to 25 mg per day on day 14 and to 20 mg per day on day 21. All patients received an oral pre-operative dose of azathioprine of 3 mg/kg, followed by an oral maintenance dose of 2–2.5 mg/kg per day, starting on day 1 post-transplant.

Tacrolimus monitoring

Whole-blood samples, used for the determination of 12 h tacrolimus trough concentrations, were collected on days 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 post-transplant and on an ad hoc basis deemed clinically necessary by the physician. Blood for trough concentration measurements was collected before the morning dose. Tacrolimus concentrations were determined at a single centre (Princess Alexandra Hospital, Brisbane) by ELISA [19].

Clinical parameters

Rejection was classified as one of two types, biopsy-proven acute rejection or presumed rejection. In clinical practice suspected rejection is not always confirmed by biopsy, but is sometimes treated empirically, due to the risk associated with performing a biopsy. Biopsy-proven acute rejection was diagnosed from renal biopsy findings scored according to the BANFF criteria [20] and recorded in the trial records.

A rejection episode was suspected clinically if there was a rise in serum creatinine of more than 30 mmol/l without obvious cause. Obstruction was ruled out with ultrasound examination. Clinical signs of rejection included fever (temperature of more than 37.5°C), graft tenderness, and oliguria. The BANFF criteria for scoring renal biopsies for acute rejection were: Normal or ‘Other’; Borderline changes; Mild Acute Rejection (Grade 1); Moderate Acute Rejection (Grade 2); Severe Acute Rejection (Grade 3). Other (Tacrolimus) Toxicity; ‘Other’ Acute Tubular Necrosis; and Chronic Transplant Nephropathy. Patients in this study were considered to have biopsy-proven rejection if according to the BANFF criteria they had Grade 1, 2 or 3 Acute Rejection. Presumed rejection was defined as a rise in serum creatinine of more than 30 mmol/l with clinical signs of rejection, treated with high-dose methylprednisolone over 3 days without biopsy, and for which no other clinical explanation was recorded in the trial results. For rejectors, median trough tacrolimus concentration was determined from the time of transplantation to the day of biopsy-proven rejection (in biopsy-proven rejecters) or the day of initiation of intravenous methylprednisolone (in presumed rejecters). For non-rejecters, median trough concentration was determined from the time of transplantation to day 30 post-transplant.

Other clinical information was collected on the type of transplant patients received (cadaveric or live related), whether it was the patient’s first or second kidney transplant, HLAA, HLAb and DR matching, peak PRA, and cold ischaemia time.

Statistical analysis

The median trough concentrations of tacrolimus in biopsy-proven acute rejectors vs non-rejectors, and all rejectors vs non-rejectors were compared. Similarly, information on transplant type, mismatching, peak PRA and cold ischaemia time was compared between all rejecters and non-rejecters. Statistical significance between two groups was determined using Student’s t-test. Alternatively, where the data failed the Kolmogorov–Smirnov normality test or the Levene median equal variance test, the Mann–Whitney rank sum test was performed (Sigmastat, Jandel Scientific Software, San Rafael, CA, USA). Probability values < 0.05 were considered to be statistically significant.

Results

A summary of the demographic, pharmacokinetic and outcome data collected in this study is presented in Table 1. One patient was excluded from investigation because oral prednisolone therapy was not initiated

| Table 1. Summary of the demographic, pharmacokinetic, and outcome data collected in this study |
|-----------------|-----------------|-----------------|
| Number of patients | 29              |
| Age              | 47 ± 13 years (19–69 years) |
| Gender           | 19 male, 10 female |
| Total samples    | 349             |
| Trough concentration | 9.83 ± 5.73 ng/ml (1.4–31.8) |
| Samples per subject | 12 ± 7 samples (1–23) |
| Biopsy-proven rejecters | 5          |
| Presumed rejecters          | 7              |
| Non-rejecters            | 17             |
until day 7 post-transplant. Of the remaining 29 patients, 19 were male and 10 female, with the median age for the cohort of 47 years (range 19–69 years). Twenty-four patients received cadaveric organs and five patients an organ from a live donor related to the recipient. It was the first kidney transplant for all patients. The mean HLAa mismatch was 1.52, the mean HLAb mismatch was 1.52, the mean HLA-DR locus mismatch was 1.31, the mean peak PRA was 16.3%, and the mean cold ischaemia time was 14 h. There were no significant differences between patients experiencing rejection and those rejection free for any of the above patient parameters (P > 0.05, Table 2).

In total, 349 trough blood concentrations were collected and analysed for tacrolimus. The number of tacrolimus concentrations measured per patient ranged from 1 to 23 with a mean (±SD) of 12 (±7) concentrations. Tacrolimus concentration ranged from 1.4 to 31.8 ng/ml (mean value (±SD) of 9.8 (±5.7) ng/ml), with most concentrations (75.4%) between 5 and 20 ng/ml.

In the study group, five patients were diagnosed as having biopsy-proven rejection, seven patients presumed rejection, and 17 patients no rejection. A mean number of 4.2 samples (range 1–6) per patient were collected from the biopsy-proven rejecters, a mean of 8.6 samples (range 5–15) per patient from the presumed rejecters and a mean of 15.8 samples (range 6–23) per patient from the non-rejecters.

On examination of the data it appeared that one of the presumed rejecters was a likely outlier with a median trough concentration (23.9 ng/ml), nearly three times the average median trough concentration in this study (8.3 ng/ml). With inclusion of the outlier in analysis the data failed a Kolmogorov–Smirnov normality test. A Mann–Whitney test was performed to determine whether a statistically significant difference existed between the different outcome groups. Median trough concentration of tacrolimus were found to be significantly lower in biopsy-proven rejecters vs non-rejecters (P = 0.03) and all rejecters vs non-rejecters (P = 0.04) (Figure 1). The average median concentration (±SD) in the biopsy-proven rejecter group was 5.09 ± 1.16 ng/ml, compared to 9.20 ± 3.52 ng/ml in the non-rejecter group. After exclusion of the outlier patient, the average median concentration in all rejecters was 5.57 ± 1.47 ng/ml, compared to 9.20 ± 3.52 ng/ml in the non-rejecter group. There was also a statistically significant difference between the last recorded tacrolimus concentration prior to diagnosis of rejection, in biopsy-proven rejecters (P = 0.02) and all rejecters (P = 0.04), compared to the average median concentration in the non-rejecter group. The last recorded tacrolimus concentration, in biopsy-proven rejecters and all rejecters (±SD) was 5.0 ± 1.38 ng/ml and 6.25 ± 3.65 ng/ml respectively, compared to an average median concentration (±SD) of 9.20 ± 3.52 ng/ml in non-rejecters.

The data were further analysed by stratification into three groups: those with median trough concentrations between 0 and 5 ng/ml, those with median concentrations between 5 and 10 ng/ml, and those with median concentrations between 10 and 15 ng/ml (Figure 2). Of patients with a median trough concentration between 0 and 5 ng/ml, 57.1% experienced presumed or biopsy-proven rejection. Of those with median trough concentrations between 5 and 10 ng/ml, 53.8%

![Fig. 1. Median trough tacrolimus concentration in each patient according to rejection status.](image)

**Table 2. Comparison of transplant type and mismatch status in rejecters vs non-rejecters**

<table>
<thead>
<tr>
<th></th>
<th>All rejecters (n = 12)</th>
<th>Non-rejecters (n = 17)</th>
<th>Total (n = 29)</th>
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<tbody>
<tr>
<td>Cadaveric transplant</td>
<td>9</td>
<td>15</td>
<td>24</td>
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</tr>
<tr>
<td>Live transplant</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>0.56a</td>
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<td>HLA mismatch (A)</td>
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<td></td>
<td>Median 2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>HLA mismatch (B)</td>
<td>Mean 1.58</td>
<td>1.47</td>
<td>1.52</td>
<td>0.72a</td>
</tr>
<tr>
<td></td>
<td>Median 2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DR mismatch</td>
<td>Mean 1.42</td>
<td>1.24</td>
<td>1.31</td>
<td>0.66a</td>
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<tr>
<td></td>
<td>Median 1.5</td>
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<td>1</td>
<td></td>
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<tr>
<td>Peak PRA (%)</td>
<td>Mean 12.4</td>
<td>19</td>
<td>16.3</td>
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<tr>
<td></td>
<td>Median 6.5</td>
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<td>7</td>
<td></td>
</tr>
<tr>
<td>Cold ischaemia time (h)</td>
<td>Mean 13.2</td>
<td>14.5</td>
<td>14.0</td>
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<tr>
<td></td>
<td>Median 13.5</td>
<td>13.1</td>
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</table>

*a*Mann–Whitney rank sum test between all rejecters and non-rejecters. *b*Student’s t-test between all rejecters and non-rejecters.
experienced presumed or biopsy-proven rejection. No patients with a median trough concentration between 10 and 15 ng/ml experienced presumed or biopsy-proven rejection.

Discussion

Most rejection episodes occur during the first month after transplant, and acute rejection appears to be a major factor in determining long-term graft outcome [21,22]. These data suggest that the risk of acute rejection is higher when tacrolimus trough concentrations are below 10 ng/ml during the early postoperative period. From this study it appears that early identification of patients with low trough concentrations and subsequent increase of their dosage is an important part of optimizing tacrolimus therapy at a time when patients are most vulnerable to acute rejection.

To date, four studies, two in liver transplant recipients [13,17] and two in kidney transplant recipients [14,12], have reported a correlation between tacrolimus trough concentrations and rejection episodes. No statistics were published in two of these studies to validate their conclusions (Takahara et al.’s study having only six subjects [14]). Winker et al. [17] conducted a large study involving retrospective analysis of 16,000 blood samples obtained from 449 patients receiving tacrolimus. A low trough concentration of tacrolimus in blood, but not plasma was associated with graft rejection in the liver transplant recipients within the study group. Kershner and Fitzsimmons [12] conducted an analysis of the relationship between tacrolimus blood concentration and the occurrence of toxicity and rejection from data collected from four clinical trials. Trough tacrolimus concentrations within a 7-day window before the onset of rejection were analysed using logistic regression models. In kidney (n = 92) but not liver recipients (n = 721) a significant correlation between tacrolimus concentrations and incidence of rejection was seen (P = 0.02). One study involving 66 kidney recipients has reported that mean area under the curve measurements of tacrolimus are significantly lower in patients who experience acute rejection compared to those who remain rejection free [15].

Other studies have shown a lack of correlation between plasma and whole blood concentrations and rejection episodes [8–11,18]. In these investigations tacrolimus concentrations were not low in patients experiencing acute rejection, but rather in the same range as during stable patient course and development of drug toxicity.

In several studies to date, the criterion for recognition of organ rejection has not been provided. Some studies have been multi-centre with the possibility of non-comparable tacrolimus assays and immuno-suppressant regimens being employed at different sites. Other factors that may influence rejection, such as type of organ received or antigen mismatching, have not been considered to ensure they are not confounding results.

Although this is a retrospective study and patient numbers are limited, results obtained were statistically significant and clinically relevant. Patients were well matched for type of transplant, HLAa, HLAb, DR, peak PRA, and cold ischaemia time, factors that can influence the likelihood of rejection. Furthermore as all patients in this study were participating in a larger clinical trial their post-transplant care and concomitant drug therapy was reasonably controlled and similar. Patients received the same initial dose of tacrolimus and were on the same dosing protocol for prednisolone, azathioprine, and methylprednisolone therapy. All patients were cared for at the same hospital and all blood samples collected were measured at the same centre using ELISA.

On examination of the data it appeared that one of the presumed rejecters was an outlier, with a median trough concentration (23.9 ng/ml), nearly three times the average median trough concentration in this study (8.3 ng/ml). As this patient’s rejection was never biopsy proven, it is possible that the rise in serum creatinine that the subject experienced was due to nephrotoxicity rather than rejection. Indeed, several past studies have demonstrated a correlation between high tacrolimus concentrations and nephrotoxicity [8,9,11,14,16–18]. If this outlier is excluded from analysis, the mean of the median trough concentrations recorded in all rejecters is 5.57 ng/ml compared to 9.20 ng/ml in non-rejecters. When only patients with biopsy-proven rejection are considered, the statistical difference between the two groups is even stronger, with average median concentration in the biopsy-proven rejecter group of 5.09 ng/ml, compared to 9.20 ng/ml in the non-rejector group.

Of trough concentrations collected in this study, 75.4% were in the reported therapeutic range for tacrolimus of 5–20 ng/ml, while 39.0% of trough concentrations were in the local hospital range of 10–20 ng/ml. Patients who experienced rejection tended to suffer their first rejection episode within the first 1–2 weeks after transplant at a mean of 8.2 days (range 5–19 days). These findings indicate that to minimize graft rejection in the first month after renal
transplant, tacrolimus trough concentrations of at least 10 ng/ml should be achieved as quickly as possible.

Acknowledgements. This work was supported financially by a grant funded by the NMHRC (#9937126). Christine Staatz received an Australian Postgraduate Award and a Baillieu Research Scholarship. The authors would also like to acknowledge the assistance of physicians, the transplant team and the Department of Clinical Pharmacology at The Queen Elizabeth Hospital in Adelaide (especially Dr Tim Mathew, Mrs Christine Russ, and Dr Ray Morris) and the pharmaceutical company Janssen-Cilag for granting permission to access clinical data.

References


Received for publication: 18.11.00
Accepted in revised form: 4.5.01