A randomized controlled trial of immunosuppression conversion for the treatment of chronic allograft nephropathy

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Abstract

Background. This study was conducted to assess the effect of immunosuppression conversion on progression of chronic allograft nephropathy (CAN).

Methods. Forty-two cyclosporin-treated renal transplant recipients were studied. Patients were included if they had a negatively sloping reciprocal of creatinine vs time (ROCT) plot for >6 months and biopsy-proven CAN. Patients were excluded if they had previously been treated with tacrolimus/mycophenolate mofetil (MMF) or their serum creatinine was >400 μmol/l.

Subjects were randomly treated with either: (A) MMF/reduced dose cyclosporin [MMF for azathioprine 0.5–1.0 g bd; cyclosporin trough level (C₀): 75–100 ng/ml]; (B) tacrolimus for cyclosporin (C₀: 5–10 ng/ml); or (C) continuation of standard therapy. Glomerular filtration rate (GFR) was measured at baseline and after 6 months.

Results. Two patients started dialysis within 6 months (one each from groups A and B). One patient in group A was intolerant of MMF, six others reported gastrointestinal symptoms and three developed anaemia. Cyclosporin dose was reduced by 24% [interquartile range (IQR): 14–27%] in group A [end-of-study C₀: 99 ng/ml (IQR: 90–113 ng/ml)]. In group B, the end-of-study tacrolimus C₀ was 7 ng/ml (5–9 ng/ml). The end-of-study cyclosporin C₀ in group C was 163 ng/ml (145–215 ng/ml). Comparison of ROCT slopes before and after intervention revealed a treatment advantage for group A (P<0.05). The GFR analysis was supportive (P=0.05). When patients with GFR <20 ml/min/1.73 m² at enrolment were excluded from the analysis, the treatment advantage for group A reached statistical significance (n=27, P<0.05).

Conclusions. MMF/reduced dose cyclosporin is superior to tacrolimus-for-cyclosporin and standard dose cyclosporin in patients with CAN, at least in the short term. The cyclosporin dose reduction component is likely to be of particular importance. Other findings suggest that early intervention is beneficial.

Keywords: chronic allograft nephropathy; cyclosporin; glomerular filtration rate; mycophenolate mofetil; randomized controlled trial; tacrolimus

Introduction

Although the early outcome of renal transplantation has continued to improve due to a number of factors, including advances in immunosuppressive therapy, there has been relatively little progress in improving long-term outcome. The 5 year survival rate for renal allografts in the UK is ~70% [1]. Chronic allograft nephropathy (CAN) is the most common cause of late transplant failure. CAN is a clinicopathological diagnosis that applies when there is a slow and progressive decline in renal transplant function in association with a range of histopathological abnormalities of glomeruli, blood vessels and graft interstitium. There are many aetiologies of CAN, some immunological and some non-immunological in nature. End-stage renal failure secondary to CAN accounts for ~3% of entrants to chronic dialysis programmes in the UK [1]. Measures to control or prevent CAN would provide valuable health and economic benefits.

Mycophenolate mofetil (MMF) is a potent and specific inhibitor of purine biosynthesis and, hence, T and B lymphocyte proliferation. There is some evidence that MMF in combination with a reduced dose of cyclosporin is an effective regimen for the treatment of established CAN [2–4]. The reported studies did not include ‘control’ patients (no change to...
immunosuppressive regimen) and glomerular filtration rate (GFR) was not measured by reference methods. In some studies there was no histological confirmation of CAN, patient follow-up was of variable duration and the use of concurrent therapies that may have altered the rate of progression of CAN [for example, angiotensin-converting enzyme (ACE) inhibitors and hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] was unreported. The majority of participants in the study by Weir et al. [3] were male African-Americans, making extrapolation of the findings to European transplant populations more problematic. The addition of MMF without concomitant cyclosporin dose reduction or withdrawal has not been beneficial in all of the reported studies, but these studies are limited also by small patient numbers, variable duration of follow-up, non-reference methods of measuring transplant function and a lack of comparative control group data [5–8].

Pilot studies of tacrolimus in place of cyclosporin for patients with CAN have been small and uncontrolled [9,10]. In one study, 5 out of 14 cyclosporin-treated renal allograft recipients who were converted to tacrolimus showed stability or improvement of function [9].

The promising results achieved in pilot studies of immunosuppression conversion for CAN need to be confirmed by larger, more rigorous studies that are prospective, randomized and controlled. The inclusion of a control group of patients whose immunosuppressive therapy is unaltered and the use of reference isotope techniques rather than serum creatinine to estimate renal function are of particular importance. We have examined two treatment regimens that may be beneficial in cyclosporin-treated patients with CAN: MMF with reduced dose cyclosporin and tacrolimus in place of cyclosporin.

Subjects and methods

The participating centres were St James’s University Hospital in Leeds (SJUH), Royal Liverpool Broadgreen University Hospital (RLBUH) and Bradford St Luke’s Hospital (BSLH). The study was planned in accordance with the World Medical Association Declaration of Helsinki, Good Clinical Practice for Trials on Medical Products in the European Union and the CONSORT guidelines [11]. Local ethics committee approval of the study was obtained prior to its commencement.

Scope

The electronic records of renal transplant recipients receiving follow-up care in the participating units were interrogated to identify patients with progressive allograft dysfunction, defined by a downwards sloping reciprocal of serum creatinine vs time (ROCT) plot over a minimum period of 6 months. The suitability of these patients for study entry was then assessed in clinic.

Eligibility

The main inclusion criteria for the study were as follows: age ≥18 years; cyclosporin-based immunosuppression; ≥6 months post-renal transplant with no episodes of acute rejection during the preceding 3 months; normal renal transplant ultrasound and Doppler examination; and biopsy-proven CAN. Transplant biopsy specimens were examined by a nominated histopathologist in each unit and classified according to the Banff criteria [12]. Exclusion criteria included the following: previous treatment with tacrolimus or MMF; HIV, HBV or HCV infection; serum creatinine >400 µmol/l; pregnant or breast-feeding female patients; female patients unwilling or unable to use approved contraception; significant, uncontrolled concurrent infection; and participation in another clinical trial during the previous month.

Eligible patients were provided with a leaflet containing information regarding the background to the study, the rationale for intervention, the timetable of the study, profiles of the study medications (MMF and tacrolimus) and issues of patient consent and confidentiality of study data.

Treatment groups

The randomized treatments were as follows: (A) MMF and reduced dose cyclosporin; (B) tacrolimus in place of cyclosporin; and (C) ‘no change’ (continuation of a cyclosporin-based immunosuppressive regimen). The protocol for each is summarized below.

**MMF and reduced dose of cyclosporin**

(i) MMF 500 mg bd commenced and azathioprine discontinued at enrolment.
(ii) MMF increased to 750 mg bd and cyclosporin dose reduced by 25% at the beginning of week 2.
(iii) MMF increased to 1000 mg bd and cyclosporin dose adjusted to achieve a trough blood level of 75–100 ng/ml (monoclonal assay; Abbott Diagnostics, Delkenheim, Germany) at the beginning of week 3.
(iv) In case of bone marrow suppression, MMF was reduced as per the following regimen: (a) if blood white cell count (WCC) was 3–4 x 10^9/ml, MMF dose was reduced to 750 mg bd; (b) if blood WCC was 2–3 x 10^9/ml, MMF dose was reduced to 500 mg bd; (c) if blood WCC was <2 x 10^9/ml, MMF was discontinued for 10 days and restarted at 500 mg bd if WCC was >4 x 10^9/ml; and (d) if neutropenia was recorded for >2 weeks, the patient was withdrawn from the study.

**Tacrolimus in place of cyclosporin**

(i) Cyclosporin discontinued at enrolment and tacrolimus started at a dose of 0.1 mg/kg/day 12 h after the last dose of cyclosporin or 24 h if the cyclosporin trough level was >300 ng/ml.
(ii) Target whole blood trough level of 5–10 ng/ml (Tacro assay; Abbott Diagnostics, Delkenheim, Germany) maintained during the study period.

**Control group**

No change to treatment regimen, target cyclosporin whole blood trough levels maintained as per unit protocol during the study period.
Randomization

Patients were allocated randomly to one of the three treatment groups using a computer-generated sequence. Information regarding the randomized treatment was concealed in sequentially numbered, sealed opaque envelopes. These were opened in the presence of the patient (by J.S. at SJUH/BSLH and G.O. at RLBUH) immediately after obtaining informed, written consent for participation in the study. Both patient and physician were necessarily aware of the randomized treatment in all cases, but members of staff in the various clinical laboratories (including Medical Physics) were blinded to this information.

Investigations

Baseline investigations included height, weight, blood pressure, GFR (99mTcDTPA-GFR), full blood count, renal biochemistry, glucose, HbA1c, fasting lipids, cyclosporin trough level and urine microscopy, culture and sensitivity. 99mTcDTPA-GFR measurement comprised upper limb intravenous injection of a small volume of solution containing 99m technetium of known radioactivity bound to DTPA and blood sampling from the contralateral upper limb at 2, 3 and 4 h post-injection. The degree of residual radioactive signal present in these samples was plotted on a decay curve, from which crude GFR was calculated and subsequently adjusted for body surface area using the Dubois formula [13]. All GFR measurements were performed in the mid-morning to early afternoon period.

Dosing and administration of additional medication

All treatments that were commenced during the study period were recorded, including dose and indication. ACE inhibitor and HMG-CoA reductase inhibitor (statin) medications were not added to patient treatment regimens as per study protocol.

Safety assessment

Patients were evaluated for adverse events following enrolment. An adverse event was defined as any untoward medical occurrence. A clinical examination and laboratory safety tests were performed at each clinic visit.

Study schedule

Patients were reviewed weekly for the first month, fortnightly for the second month and monthly thereafter. A second 99mTcDTPA-GFR measurement was performed at the end of the 6 month study period.

Primary study outcome measure

The primary outcome measure was change in renal function. This was assessed by comparing 99mTcDTPA-GFR values at the start of the study and at its conclusion 6 months later and the slope of ROCT plots before and after treatment intervention.

Secondary study outcome measures

Hypertension and hypercholesterolaemia, two important side effects of immunosuppressive therapy, were selected as the secondary study outcome measures. Change in urine protein creatinine index (PCI) was also assessed.

Power of study

There is a variable rate of functional decline in some patients with CAN, such that slowing of disease progression may be independent of the therapeutic intervention. Assuming a SD of 4 ml/min/1.73 m² for the annual reduction in GFR in patients with CAN, a study population of 48 patients would be required for a 3 ml/min/1.73 m² per year difference between treatment groups to be statistically significant ($P < 0.05$, power 80%).

Statistical methods

The difference in slope of the pre- and post-intervention ROCT plots was calculated for each patient and an intergroup comparison of values was made using a Kruskal–Wallis test. It was not assumed that pre- and post-study slopes would have a common intercept at time zero. Intergroup comparisons of changes in GFR, cholesterol, triglycerides and systolic and diastolic blood pressure between the start and finish of the study were also made using a Kruskal–Wallis test. Analysis of covariance (ANCOVA) was performed to assess the relevance of the pre-study GFR to therapeutic response.

Interim analysis

An interim analysis of data was conducted after 36 patients had completed the study (75% of the required number) by a statistician who was otherwise uninvolved with the research. There were two main reasons for not performing the analysis sooner. Firstly, the differences in outcome between the three treatment groups were not expected to be large. Secondly, the study was of short duration and it was felt that the initiation of a more effective treatment (if this were to be proven) for a chronic pathology could be deferred without major consequences. The analysis was reviewed by senior clinicians who had been involved in the planning, but not the execution, of the study.

The study sponsors had no involvement in the study design, the collection, analysis and interpretation of data, the writing of the report and the decision to submit it for publication.

Results

The first study patient was enrolled in July 1999. The study was completed (last patient out) in June 2002. Forty-two patients entered the study, less than the target number derived from the power calculation. This was because of a slow take-on rate towards the end of the recruitment period (December 2001).

There were no differences between the three study groups at baseline that were of statistical or clinical significance. A patient flow chart for the study participants is shown in Figure 1. Two patients started dialysis treatment during the 6 month study period, one in each of the intervention groups. In both cases
Fig. 1. A flow chart for study participants.
the initial GFR was <20 ml/min/1.73 m² and there was evidence of a rapid pre-study decline in graft function. An end-of-study GFR of 5 ml/min/1.73 m² was assumed for both patients in the main GFR analysis. Two patients failed to attend for an end-of-study GFR measurement (a control group patient and a patient who appeared to respond well to a MMF/reduced dose cyclosporin regimen according to serum creatinine values) and GFR was not measured in another control group patient because of her needle phobia. These patients were, therefore, not included in the GFR analysis.

One patient was intolerant of MMF and withdrew from the study after a few days of treatment. His data were disregarded as we wished to examine the ‘on-study’ effectiveness of treatments rather than analyse outcomes on an ‘intention-to-treat’ basis.

Cyclosporin dose was reduced by a median of 24% [interquartile range (IQR): 14–27%] in the group receiving MMF, giving a median end-of-study cyclosporin trough blood level of 99 ng/ml (IQR: 90–113 ng/ml). The median maintenance dose of MMF was 1.5 g/day (IQR: 1.5–2 g/day). The median end-of-study tacrolimus trough blood level in the group receiving tacrolimus was 7 ng/ml (IQR: 5–9 ng/ml). The median end-of-study cyclosporin trough blood level for control group patients was 163 ng/ml (IQR: 145–215 ng/ml).

ROCT plots were constructed for each patient, taking creatinine data from 12 months before to 6 months after study entry. During the ‘run-in’ period, serum creatinine was measured when patients attended for routine clinical review. The median number of creatinine values per patient over the ‘run-in’ period was 10 (IQR: 7–12). A comparison of change in ROCT slope revealed a significant treatment advantage for patients in group A (Kruskal–Wallis test, \( P < 0.05 \)). Dotplots for change in ROCT slope are shown in Figure 2.

The analysis of GFR data (available in 37 patients) also showed a trend towards a treatment advantage for MMF/reduced dose cyclosporin (\( P = 0.05 \); Figure 3). The median increase in GFR in the MMF/reduced cyclosporin group was 2.5 ml/min/1.73 m² compared with a median decrease of 0.7 ml/min/1.73 m² in the control group. Figure 4 shows the correlation between change in GFR and the slope of the ROCT plot between 0 and 6 months (\( r = 0.61, P < 0.001 \)). An ANCOVA model including GFR at the start of the study (GFR0) as a covariate showed that this was a significant predictor of response to treatment (\( P < 0.05 \)). To explore this further, patients with poor initial transplant function (GFR <20 ml/min/1.73 m²) were excluded from a repeat analysis. This showed a similar but more exaggerated trend towards a treatment advantage for MMF/reduced dose cyclosporin [median GFR increase of 3.3 ml/min/1.73 m² compared with a median decrease of 0.7 ml/min/1.73 m² in the control group (\( n = 27, P < 0.05 \); Figure 5)].

The changes in fasting lipid profile, arterial blood pressure and proteinuria during the course of the study are summarized in Table 1. Blood pressure reduction was more substantial in the intervention groups, but this did not reach statistical significance. There was also a non-significant trend towards an improvement in the serum lipid profile of patients receiving tacrolimus as compared with controls. There were no significant differences between treatment groups at the start and at the end of the study period in terms of the number of prescribed antihypertensive agents. Change in urine PCI during the 6 months study period in the three treatment groups was not significantly different.

In the MMF/reduced dose cyclosporin group, gastrointestinal disturbance occurred in six cases. This generally improved with MMF dose reduction. Two patients reported insomnia. One patient required treatment for a recurring external ear infection. MMF
dose was reduced in three patients because of progressive anaemia. One patient in the tacrolimus group developed progressive hair loss and her treatment was discontinued shortly after study completion. Another patient receiving tacrolimus required treatment for acne. Of the patients in the control group, one required surgical drainage of a perianal abscess, one developed acute gout and another required treatment for a urinary tract infection. None of the study patients developed acute rejection or de novo diabetes mellitus during the study period.

Discussion

This is the second prospective randomized controlled trial of immunosuppression conversion for cyclosporin-treated renal transplant recipients with established CAN. The Creeping Creatinine Study [14] examined the effect of introducing MMF followed by complete withdrawal of cyclosporin and was unique in having a control group. Renal function stabilized in 58% of patients receiving MMF in place of cyclosporin (n = 73) as compared with 28% of controls (n = 70). The incidence of acute rejection in the two groups was similar. Three deaths were reported in the MMF group during a follow-up period of ~9 months.

Our study provides evidence of a treatment advantage for MMF/reduced dose cyclosporin as compared with substitution of tacrolimus for cyclosporin and standard dose cyclosporin in patients with established CAN, at least in the short term. A reference method of GFR measurement (99mTcDTPA-GFR) was used to monitor change in transplant function in addition to...
more frequent serum creatinine measurements. The 99mTcDTPA-GFR method was performed only twice (at the start of the study and 6 months later). ROCT plots gave more information about pre-study patterns of functional attrition. Analyses of GFR and creatinine data gave similar results. It would have been preferable for the number of study participants to at least equal the power calculation estimate, but this could not be achieved within the time limits of the study. It can be seen that for control patients the decline in renal function according to ROCT plots was a little slower during the study period as compared with the ‘run-in’ period (Figure 2). This may have been due to a number of factors, such as improved clinical management of patients during the study period and the inherently variable rate of disease progression that has been reported in CAN [15]. These points serve to emphasize the importance of including a control group in randomized studies.

The apparent success of the above regimens could be explained simply in terms of the known effect of cyclosporin on renal haemodynamics [16] and pro-fibrotic cytokine expression [17]. MMF is effective in reversing some of the histopathological features of CAN in the rat [18] and therapeutic concentrations of the drug inhibit fibroblast growth in cell-culture studies [19]. There is also some evidence that the use of MMF in place of azathioprine produces clinical benefit in terms of a reduced incidence of chronic renal allograft failure [20]. Although the main aim of the study was to study the safety and efficacy of immunosuppression conversion for CAN rather than the mechanism by which benefit might be achieved, we did perform a post-hoc analysis comparing data from ‘1 year before to the start of study’ and data from ‘3 months to 1 year’ to determine whether the response to MMF/reduced dose cyclosporin was attributable to a step change in GFR following cyclosporin dose reduction or this and a continuing protective effect of MMF. Our basic assumption that the acute GFR response to cyclosporin dose reduction occurs within 3 months is supported by data from Dudley et al [14]. We found a non-significant improvement in ROCT slope for the 3 months to 1 year period compared with the ‘run-in’ period \((P = 0.08)\). The body of follow-up data at 12 months was less complete than at 6 months, because of graft loss in a

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**Table 1. Change in blood pressure, serum lipids and urinary protein excretion (pre-study minus post-study values) in the three treatment groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>MMF/reduced dose cyclosporin</th>
<th>Tacrolimus in place of cyclosporin</th>
<th>Continuation of cyclosporin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>6 (–8–22)</td>
<td>10 (–10–22)</td>
<td>5 (–10–20)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>10 (1.5–15)</td>
<td>7 (0–11)</td>
<td>3 (–2–15)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>0.3 (–0.7–0.9)</td>
<td>0.9 (0.1–1.4)</td>
<td>0.4 (–0.1–0.8)</td>
</tr>
<tr>
<td>Low density lipoprotein cholesterol (mmol/l)</td>
<td>–0.4 (–1–0.4)</td>
<td>0.6 (0.1–1)</td>
<td>0.1 (–0.2–0.2)</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol (mmol/l)</td>
<td>0 (–0.1–0.3)</td>
<td>0.1 (0–0.2)</td>
<td>0 (–0.2–0.1)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.1 (–0.3–0.2)</td>
<td>0 (–0.3–0.8)</td>
<td>0.2 (–0.3–0.5)</td>
</tr>
</tbody>
</table>

Data are medians with IQR in parentheses.
control patient and discontinuation of tacrolimus in a patient who developed progressive hair loss.

The importance of intervening at a relatively early stage in the development of CAN is highlighted by two separate findings of this study. Firstly, baseline GFR (GFR0) was found to be a significant predictor of outcome in an ANCOVA model with GFR0 as the covariate. Secondly, an analysis of change in GFR that excluded patients with a GFR0 of <20 ml/min/1.73 m² revealed a more definite difference in outcome between the treatment groups. These findings emphasize the need for prompt detection of CAN.

The rate of change of GFR was reduced in some patients receiving tacrolimus, but the treatment response in the tacrolimus group as a whole was not superior to controls. The characteristics of patients who appeared to respond well were not different to those of others in the group. There was a non-significant trend towards an improvement in the serum lipid profile of tacrolimus-treated patients as compared with controls.

In summary, this randomized controlled comparative study of immunosuppression regimens in patients with established chronic allograft nephropathy provides evidence to support the use of MMF and low-dose cyclosporin in preference to a standard dose cyclosporin-based regimen. More substantial reductions in cyclosporin dose might produce an even better outcome, as this component of the regimen appears to have the greatest impact on graft function, at least in the short term. However, the increased risk of acute graft dysfunction associated with total cyclosporin withdrawal needs to be considered [21]. Further studies to compare MMF-based regimens with newer immunosuppressive agents, such as rapamycin, might help to determine an optimal regimen for the prevention and treatment of CAN. Timely intervention depends on early detection of CAN. This is probably best achieved by protocol transplant biopsy as there are currently no validated non-invasive tests of renal allograft injury. This is probably best achieved by protocol transplant biopsy as there are currently no validated non-invasive tests of renal allograft injury.

References


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