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ORIGINAL ARTICLE

Cyclosporine dose reduction in stable renal transplant patients with high C2 level: simplified method of single C2 measurement and individualization of C0 target

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Summary

It is recommended that cyclosporine dosing should be based on the whole blood level 2 h after a dose (C2), not the trough level (C0). Initial studies did not however establish the outcome of dosing according to C2 levels in long-term patients previously managed by C0 levels. C0 and C2 were measured in 152 stable patients receiving Neoral therapy, mean 86.9 months after transplantation. This showed that 38 (25%) had C2 levels above a target range of 700–900 μg/l. Higher C2 levels were associated with higher cholesterol levels ($P = 0.0058$) and higher diastolic blood pressure ($P = 0.0163$). Cyclosporine dose reduction was undertaken in 32 patients with high C2 levels. For logistical reasons, C2 was not performed regularly, but an individualized C0 level was set for each patient. A 16% reduction in mean cyclosporine dose was achieved, associated with a 28% fall in mean C0, from 212 to 153 μg/l, and a 25% fall in mean C2, from 1075 to 820 μg/l. There was no excess in adverse events in the dose reduction cohort, compared with patients with initial C2 levels <900 μg/l. Over a mean 15 month follow-up period in the dose reduction cohort, there was a 4.4% reduction in mean diastolic blood pressure, from 84.9 (SEM 2.1) to 80.2 (1.9) mmHg, $P = 0.023$; and a 10.4% reduction in mean cholesterol, from 5.71 (0.27) to 5.11 (0.25), $P = 0.005$ (patients starting on statin during follow-up excluded). In patients with initial C2 <900 μg/l, blood pressure did not fall and the cholesterol fell by 3.9%, from 5.27 (0.14) to 5.07 (0.15) mmol/l ($P = 0.0405$). In conclusion, cyclosporine dose reduction was safe in stable long-term renal allograft recipients with high C2 levels. There was an improvement cholesterol levels and a small improvement in blood pressure after cyclosporine dose reduction.

Introduction

Patients receiving cyclosporine, in the maintenance phase after renal transplantation, have conventionally been monitored according to the trough level of the drug (C0). More recently it has been recognized that when using the Neoral preparation of cyclosporine, monitoring by the blood level 2 h (C2) after dose administration is associated with better outcomes early after transplantation. This is because the immunosuppressive effect of cyclosporine is associated with the area under the concentration-time curve (AUC), and that the blood level at a single time point best correlated with the AUC is at C2, not C0 [1–5].

Although studies performed in the early post-transplant period showed an improvement in acute rejection rate with C2 monitoring, there were no published long-term studies when the UK data sheet produced by Novartis recommended that Neoral be monitored by C2 levels, with a target of 'around' 800 μg/l.

The aim of this study was, first, to measure the C0 and C2 in our maintenance patients receiving cyclosporine, and to see if these levels were associated with the clinical status of the patients. Secondly, we wished to adjust the cyclosporine dose for those patients who had C2 levels above target, and to report the safety and short-term benefits of this change.

It was decided not to change to routine C2 monitoring. There were several reasons for this. First, there was no proven benefit for making this change. Secondly, our patients have blood taken in a variety of primary care settings and different hospital phlebotomy clinics before transplant outpatients. Many of these facilities are busy with a queue of patients waiting for various blood tests. Therefore, changing everyone to C2 monitoring, when blood should be taken within 15 min of the 2 h post-ingestion time, did not seem practical. This was especially so when tacrolimus and sirolimus blood levels continue to be measured at C0.

Patients and methods

Stable patients had C0 and C2 cyclosporine levels measured in the transplant clinic. Staff observed the administration of cyclosporine and took blood exactly 2 h later. Renal function, fasting lipids and blood pressure were also measured.

The target C0 level was 125–225 μg/l. Given a recommended target C2 level of 'about 800 μg/l' in maintenance patients, we took 700–900 μg/l as our C2 target range. Patients with a C2 level above 900 μg/l then had clinical review to see if cyclosporine dose reduction was appropriate. If so, a new target C0 level was assigned. Initially this was carried out by strict mathematical dose reduction (e.g. if the C2 level was 25% too high, a 25% dose reduction), but our experience suggested that the level was likely to fall to a proportionately greater extent than the dose reduction in some patients, so individualized C0 targets were approached more cautiously. After any dose reduction, the C0 and serum creatinine were measured within 2 weeks, and further dose modification performed if necessary. At a mean of 15 months follow-up, the dose reduction cohort underwent further C0 and C2 testing.

Cyclosporine levels were measured by the Abbott assay (Maidenhead, UK). Just before C0 and C2 were due to be repeated, the laboratory in our center changed the assay method to Roche Diagnostics (Basel, Switzerland). The Roche assay gives results lower then the Abbott assay (−14%, −14 μg/l by local quality control). It was not felt appropriate to convert Roche assay results to an assumed Abbott level. Therefore C0 and C2 repeat levels were performed using an Abbott assay in another laboratory for the dose reduction cohort only. C0 and C2 levels measured using the Roche assay are not reported here.

Statistical anlaysis was performed using paired Student's *t*-test, Pearson's correlation coefficient, and sign testing as appropriate.

Results

The characteristics of 152 patients who underwent C0 and C2 monitoring are shown in Table 1. The range between transplantation and monitoring was 5–227 months. Sixty (60.5%) patients were taking cyclosporine, azathioprine and prednisolone; 35 (23%) were taking cyclosporine and prednisolone; 13 (8.6% were taking cyclosporine and azathioprine; four (4.6%) were on cyclosporine monotherapy; five (3.3%) were on cyclosporine and mycophenolate, three with prednisolone. Baseline lipids, taken on the same day as C0 and C2, were only available in 127 of the patients. The distribution of C0 and C2 levels according to the target ranges is shown in Table 2, and correlations between C0, C2 and various clinical parameters are shown in Table 3. The mean time from transplantation to testing was 59.6 (SEM 7.9) months in the group with C2 >900 ng/ml; 92.7 (6.7) months in the C2 700–900 group; and 100

Table 1. Characteristics of patients undergoing C0 and C2 measurement [mean (SEM)].

Number	152
Male:female	91:61
Age (years)	49.1 (1.22)
Time since transplant (months)	86.9 (4.3)
Cyclosporine dose (mg/day)	231 (6)
C0 (μg/l)	177 (4)
C2 (μg/l)	786 (18)
Serum creatinine (μmol/l)	148 (4)
Cholesterol, fasting (mmol/l)	5.58 (0.10)
Triglycerides, fasting (mmol/l)	2.06 (0.10)
Patients on statins	17 (11%)
Systolic blood pressure (mmHg)	145.8 (1.6)
Diastolic blood pressure (mmHg)	81.0 (0.8)
Number blood pressure (drugs/patient)	1.84 (0.08)
Urate level (mmol/l)	472 (9)

Table 2. Distributions of C0 and C2 levels.

	C2 <700 μg/l (%)	C2 700–900 μg/l (%)	C2 >900 μg/l (%)
C0 <125 μg/l	19 (13)	7 (5)	1 (1)
C0 125–225 μg/l	31 (20)	42 (28)	27 (18)
C0 >225 μg/l	3 (2)	12 (8)	10 (6)

Total number of patients (*n*) 152.

Table 3. Correlations (Pearson correlation coefficient) between C0, C2 and other parameters.

	C0	C2
C0	–	$P < 0.0001$
Cyclosporine dose	NS	$P < 0.0001$
Serum creatinine	$P = 0.0175$	NS
Cholesterol	$P = 0.0007$	$P = 0.0058$
Cholesterol (patients on statins excluded)	$P = 0.0005$	$P = 0.0015$
Triglycerides	NS	NS
Systolic blood pressure	NS	NS
Diastolic blood pressure	NS	$P = 0.0163$
Number blood pressure drugs	NS	NS
Urate	NS	NS

(7) months in the group with C2 <700 ng/l ($P = 0.0006$). This reflects a tendency to dose to higher cyclosporine levels in more recent years.

The 38 patients with a C2 level >900 ng/l were considered for cyclosporine dose reduction. In six cases, the dose was not changed for clinical reasons. These were cyclosporine monotherapy; intermittent clinic attendance; and physician caution (four cases). The other 32 cases underwent dose reduction, and we describe the safety and clinical outcome over a mean of 15.4 months (range 7–22) follow-up. Dose reduction started 3–6 months after the initial C2 monitoring.

Adverse clinical events were: C2 >900 μg/l group, one graft failed [chronic allograft nephropathy (CAN)]; 1 reversible acute renal failure; C2 <900 μg/l group, five patients died; one graft failed (CAN); five biopsies for dysfunction (one CAN; one acute rejection; three transplant glomerulopathy); one acute renal failure. Total adverse events were two of 32 (6%) in the C2 >900 μg/l group who had dose reduction, and 12 of 114 (11%) in the C2 <900 μg/l group.

Table 4 shows initial results and follow-up in patients with functioning grafts who were receiving cyclosporine. Although the mean creatinine in the both groups rose slightly, this was skewed by some patients with larger increases in creatinine because of CAN. In both groups more patients experienced a fall in creatinine than a rise (22 vs. nine in dose reduction group, $P = 0.0294$, 68 vs. 34 in the C2 in range group, $P = 0.001$, signed test). There was no significant change in blood pressure therapy in the dose reduction group, mean 1.94 (SEM 1.04) and 1.94 (0.05) agents per patient before and after dose reduction; three patients had a reduction in number of agents, two patients had an increase in number.

The mean reduction in cyclosporine dose was 44 mg/day, a 16% reduction, so that the annual cost saving was approximately £15 050 (21 800 euros) per annum in this cohort.

Discussion

The C2 monitoring was performed in maintenance phase patients treated with cyclosporine (Neoral). This showed that a quarter of patients had C2 levels above the target range, most of whom had C0 levels within, or even below, the target range for C0.

Table 4. Outcomes after follow-up, and cyclosporine dose reduction in the cohort with high Neoral C2 levels [mean (SEM)]. Significance tested by paired Student's *t*-test.

	C2 high, dose reduced		C2 in range	
	Start	At follow-up	Start	At follow-up
C0 (μg/l)	212 (12)	153 (10)*	166 (5)	ND†
C2 (μg/l)	1075 (19)	820 (33)‡	689 (15)	ND†
Cyclosporine dose (mg/day)	281 (13)	237 (13)	215 (7)	212 (7)
Creatinine (μmol/l)	161.2 (8.6)	172.7 (17.4)	144.3 (4.0)	163.6 (6.8)
Systolic blood pressure (mmHg)	150.1 (3.0)	147.7 (2.9)	143.1 (1.9)	147 (2.1)§
Diastolic blood pressure (mmHg)	84.9 (2.1)	80.2 (1.9)¶	79.8 (0.9)	81.5 (1.3)
Number of blood pressure drugs	1.94 (0.18)	1.94 (0.17)	1.85 (0.09)	1.80 (0.10)
Cholesterol (mmol/l)**	5.71 (0.27)	5.11 (0.25)††	5.27 (0.14)	5.07 (0.15)‡‡
Triglycerides (mmol/l)	1.86 (0.21)	1.64 (0.16)	2.06 (0.16)	2.07 (0.19)

*$P = 0.0003$ compared with C0 before dose reduction.
†$P < 0.0001$ compared with C2 before dose reduction.
‡Not performed (change in local assay, see text).
§$P = 0.044$, compared with systolic blood pressure at start.
¶$P = 0.023$, compared with diastolic blood pressure before dose reduction.
**Patients starting, or stopping, statin during follow-up period excluded (two in dose reduction group, nine in no change group).
††$P = 0.005$, compared with cholesterol before dose reduction.
‡‡$P = 0.041$, compared with cholesterol at start.

Exposure to cyclosporine measured by C2 levels was associated with fasting cholesterol levels. There were less strong associations between diastolic blood pressure and C2 levels, and between serum creatinine and C0 levels. Patients potentially underexposed to cyclosporine seemed clinically stable, and their characteristics did not differ from the 'in range' or 'overdosed' patients. In particular, there was no excess of graft dysfunction in any of these three groups, a finding in contrast to one study where patients with a low C2 tended to have CAN [6], but another study has shown no association between low C2 levels and graft dysfunction [7].

It was felt appropriate to reduce the cyclosporine doses in those patients who were potentially overdosed. Ultimately, 32 of the 38 patients eligible for dose reduction has their doses reduced, because of physician caution in the remaining six patients. These six patients have remained clinically stable during follow-up, although this is currently of short duration.

Marked logistic problems were apparent if we were to change a subgroup of patients to regular C2 monitoring. Most of our maintenance patients have blood taken near their homes before coming to clinic, and the demands of achieving venesection within 15 min of the 2 h postdose time seemed disproportionate. Therefore we chose to individualize the C0 target level in these patients. It could be argued that changing the dosing in this way on the basis of single C2 levels did not take account of possible intra-patient variability in blood levels. However, previous work by ourselves and others has shown that the coefficient of variability is less than 10% for C0 levels, and about 10–20% at 2 h [4,8]. Thus the risks of inadvertently underdosing patients seemed low, although it was recognized that some patients with very high C2 levels might not achieve a C2 level below 900 μg/l by this method.

In our initial dose reduction schedule, 32 patients underwent 49 dose changes to achieve a target C0, no patient having more than three dose adjustments. This resulted in 22 of patients achieving a satisfactory C2 level. Overall, the cyclosporine blood levels fell by a greater percentage than the dose reductions. A 16% reduction in mean cyclosporine dose was associated with a 28% fall in mean C0, from 212 to 153 μg/l, and a 25% fall in mean C2 level, from 1075 to 820 μg/l.

The reduction in cyclosporine dose seemed safe in this small number of patients, and it should also be noted that there was no excess of adverse events after dose reduction in the Toronto study [7]. Dose reduction was followed by improvements in creatinine (69% of subjects had a fall in creatinine), 4.4% reduction in mean diastolic blood pressure, and a 10.4% reduction in mean cholesterol levels. However, those patients with an initial C2 <900 μg/l also experienced a fall in cholesterol of 3.9%, and 60% of these subjects had a fall in creatinine levels.

It is interesting to compare our experience with that of the Toronto group, who performed a similar study, using a similar C2 target range [7]. A higher proportion of their patients had high C2 levels (48% compared with 25% in our center), but the reasons for this are not clear. In the cohort undergoing dose reduction, C2 monitoring was performed regularly in Toronto. A 26% reduction in cyclosporine dose was achieved, compared with a 16% reduction in our cohort. This may have been because regular C2 monitoring allowed reductions to be made with more confidence in the Toronto cohort. This is reflected in a smaller drop in mean C2 after 15 months follow-up. Mean C2 fell by 24%, from 1075 to 820 μg/l in our cohort, and by 36%, from by 1090 to 702 μg/l in Toronto.

Both centers noted some improvements in renal function, blood pressure, and lipids, although these were not major when compared with the control groups. The 10.4% fall in cholesterol in our series was greater than that observed in the Toronto group, but it is difficult to compare the cohorts because patients who started statins during follow-up were excluded from our analysis, and it is likely that more of the Canadian patients were on statin therapy throughout their study. In addition they had a lower mean cholesterol level at outset. Broadly, however, the outcomes of these two studies are comparable, and confirm that some patients experience benefit from cyclosporine dose reduction if they have a high C2 level.

Data on C2 monitoring from other centers for maintenance renal transplant patients are currently available in preliminary form. These broadly confirm the findings from our center and from Toronto [6,9,10].

Although C2 monitoring readily allows the identification of a proportion of patients who may be overdosed with cyclosporine, the overall benefits of reducing cyclosporine dose by 15–25% in a quarter to a half of maintenance patients are unclear. Given that studies by other workers and ourselves have shown that both donor specific responsiveness and lymphocyte sensitivity to cyclosporine vary between individuals by more than an order of magnitude [11–14], refining a pharmacokinetic technique that exposes all to the same level of immunosuppression may not be of substantial benefit. There is still a powerful rationale for developing techniques that administer immunosuppression in the context of individuals' immune responsiveness.

In conclusion, cyclosporine monitoring by measurement of C2 levels showed that 25% of our maintenance renal allograft patients were above the target range for C2. Cyclosporine doses were reduced in the majority of these patients, by a mean of 16%. Dose reduction was

safe, and resulted in some improvements in blood pressure and cholesterol levels.

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Conflict of interest

This study was not supported by external funding. The Renal Transplant Unit has previously taken part in clinical studies supported by Novartis and the manufacturers of other immunosuppressive drugs.

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