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ORIGINAL ARTICLE

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Cyclosporin withdrawal with concomitant conversion from azathioprine to mycophenolate mofetil in renal transplant recipients with chronic allograft nephropathy: a 2-year follow-up

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Abstract Because recent large studies have demonstrated that mycophenolate mofetil (MMF) is superior to azathioprine (AZA) as a post-transplant immunosuppressant, it has been speculated that MMF could have a cyclosporin (CsA)-sparing effect in renal transplant recipients with chronic allograft dysfunction. Between April 1996 and October 1998, 31 patients with chronic allograft dysfunction were assigned to have conversion from AZA to MMF with concomitant CsA withdrawal. Patient and graft outcomes were analysed. Mean follow-up time after MMF conversion was 27 ± 11 months. Serum creatinine concentration (sCt) significantly decreased after conversion and remained stable at the end of follow-up (227 ± 31 μmol/l vs. 185 ± 50 μmol/l; $P < 0.0005$). Mean variation in sCt was −24% after conversion, whereas it was +20% in the year before conversion ($P < 0.001$). There was a significant inverse relationship between proteinuria at baseline and improvement in renal function ($r = -0.35$; $P = 0.01$). Proteinuria increased during follow-up (0.79 ± 0.6 vs. 1.79 ± 1.08 g/day; $P = 0.04$). Isolated CsA nephropathy was associated with the best outcome. Renal function significantly improved in patients with grade 1 chronic rejection and remained stable in patients with grade 2 chronic rejection. Two patients (6.5%) experienced late acute rejection, respectively 13 and 24 months after CsA withdrawal. Eight patients (29%) experienced systemic infections requiring hospitalization. Blood pressure control and lipid profile improved after conversion. CsA withdrawal with a concomitant switch from AZA to MMF allows a substantial and durable improvement in renal function. Both allograft histology and proteinuria at baseline are predictive of the evolution of renal function after conversion. Physicians should consider the risk of over-immunosuppression possibly associated with this therapeutic strategy.

Keywords Renal transplantation · Mycophenolate mofetil · Cyclosporin withdrawal

Introduction

Major advances in immunosuppression, including the introduction of cyclosporin (CsA), have led to dramatic improvements in short-term renal allograft survival [7, 20]. Moreover, the projected half-life of a renal allograft has significantly increased. The main cause of graft loss remains chronic allograft nephropathy, which is due to both immunological and non-immunological factors, leading to the development of non-specific pathological features. CsA nephrotoxicity is considered to be one of the significant non-immunological factors of chronic

allograft nephropathy [3, 10]. Thus, it has been suggested that any benefit derived from the reduced incidence of acute rejection in the first year after transplantation may be more than offset by the progressive and potentially irreversible CsA-associated nephropathy.

Mycophenolate mofetil (MMF), the morpholinoethyl ester of mycophenolic acid, is a potent, reversible, uncompetitive inhibitor of inosine monophosphate dehydrogenase. MMF acts as a selective inhibitor of T- and B-cell proliferation by blocking the production of guanosine nucleotides, and interfering with the glycosylation of adhesion molecules. Because recent large studies have demonstrated that MMF is superior to azathioprine (AZA) as a post-transplant immunosuppressant [9], it has been speculated that MMF could have a CsA-sparing effect in renal transplant recipients.

Our group has recently reported that CsA withdrawal with a concomitant switch from AZA to MMF seemed to be safe and allowed a significant improvement of renal function in patients with biopsy-proven CsA nephrotoxicity [6]. Moreover, because it has been shown to inhibit intimal proliferation [1, 2], a hallmark of chronic rejection, and to delay renal failure in animal models [16, 17], it has been suggested that MMF may be useful in the treatment of chronic allograft nephropathy [11, 21]. As a consequence, we considered CsA withdrawal with concomitant switch from AZA to MMF in our patients with chronic allograft dysfunction.

We report here long-term follow-up of 31 renal transplant recipients with chronic allograft dysfunction who underwent CsA withdrawal accompanied by conversion from AZA to MMF. Renal function, tolerance, and immunological status (rejection, complications of over-immunosuppression) were studied.

Subjects and methods

Between April 1996 and April 1998, 31 patients with chronic allograft dysfunction were assigned to have conversion from AZA to MMF with concomitant CsA withdrawal. The protocol conversion was the following: AZA was stopped and MMF (2 g/day) was immediately introduced, while CsA dosage was not modified. The decrement in CsA dosage was begun 2 weeks later. CsA dosage was decreased by 25% every 2 weeks, until CsA was withdrawn 8 weeks after the beginning of MMF. Steroid dosage was not modified.

All the patient had biopsy-proven chronic allograft nephropathy according to the Banff classification.

Patients were kept on their routine anti-hypertensive medications with the purpose of maintaining a blood pressure lower than 140/90 mmHg.

Clinical (age, gender, transplant duration, blood pressure, tolerance, serious adverse events), biological (serum creatinine concentration, haemoglobin concentration, proteinuria, LDL-cholesterol, triglycerides) and histological correlates were recorded in the year before conversion, at inclusion and during follow-up. Variations in serum creatinine concentration (sCt) (sCt1–sCt2/baseline sCt1) were measured in the year before and during follow-up after conversion. Variations in sCt could range from positive values (decrease in GFR over time) to negative values (increase in GFR over time).

The last evaluation was the last visit of the patient while still on MMF and steroids alone.

Histology

Histological examination was performed in all patients in the 6 months before the conversion to MMF. CsA nephropathy was diagnosed according to the following morphological criteria: CsA arteriolopathy characterized by a ring of nodular hyaline deposits on the outer surface of the afferent arteriolar wall or non-specific arteriolar hyalinosis; a focal or striped form of interstitial fibrosis; tubular atrophy; isometric vacuolization of tubules; and interstitial infiltrates made up of lymphocytes and histiocytes, only when slight and limited to the areas of fibrosis. Acute and chronic rejection were diagnosed according to the Banff 97 classification. A second biopsy was systematically performed approximately 1 year after conversion. The same pathologist reviewed all the biopsies.

Statistics

Results are given as mean ± SD. The paired Student *t*-test was used for comparing differences between groups, and the Spearman test was used for estimating relationships between variables. The ordinal data were analysed using a chi-square test. A P value < 0.05 was considered significant.

Results

The baseline characteristics of the patients are summarized in Table 1.

Mean follow-up after MMF conversion was 27 ± 11 months.

CsA withdrawal was achieved in all patients at week 8 after the beginning of MMF.

Mean AZA dosage was 64 ± 29 mg/day. Mean MMF dosage at the end of the study was 1.6 ± 0.35 g/day.

Renal function

Serum creatinine concentration significantly decreased after conversion and remained stable at the end of follow-up (227 ± 31 μmol/l vs. 185 ± 50 μmol/l; $P < 0.0005$) (Table 2). At the end of follow-up, renal function had worsened in four patients (13%), had remained stable in two (6.5%), and had improved in 25 (80.5%). Mean

Table 1. Baseline characteristics of the study population

Characteristic	Result
n	31
Gender (m/f)	20/11
Age (years)	47 ± 11
Transplant duration (months)	66 ± 41
Previous acute rejection (%)	0.4 ± 0.3
sCt (μmol/l)	227 ± 31
U.P.E. (g/day)	0.79 ± 0.6

Table 2. Renal function, proteinuria and lipid profile before conversion and at the last evaluation

Parameter	Before conversion	Last evaluation	*P*
Serum creatinine concentration (μmol/l)	227 ± 31	185 ± 50	< 0.0005
Proteinuria (g/day)	0.79 ± 0.6	1.79 ± 1.08	0.1
Number of anti-hypertensive drugs	1.81 ± 1.12	1 ± 0.8	< 0.01
LDL-cholesterol (g/l)	1.36 ± 0.5g/l	1.21 ± 0.3	0.03
Triglycerides (g/l)	1.71 ± 0.4g/l	1.4 ± 0.3g/l	0.01

variation in sCt was –24% from baseline sCt after conversion, whereas it was +20% in the year before conversion ($P < 0.001$).

There was no relationship between the evolution of renal function and sCt, transplant duration and CsA through levels at baseline. Nevertheless, there was a significant inverse relationship between proteinuria at baseline and the improvement of renal function at the end of follow-up ($r = -0.38$; $P < 0.01$) (Fig. 1).

Patient outcomes

One patient was returned to dialysis 6 months after withdrawal of all immunosuppression because of breast cancer. At the end of follow-up, 25 patients (81%) well still alive with a functioning graft, one patient (3%) was on dialysis and five (16%) had died (cancer, two; infection, two; acute myocardial infarction, one). Among the 25 patients with a functioning graft, 24 were still on MMF.

Relationship between histology at baseline and improvement in renal function

At baseline, kidney biopsies exhibited Banff grade 1 chronic rejection in 12 patients, Banff grade 2 chronic rejection in nine, and isolated CsA nephropathy in ten. Features of CsA nephrotoxicity were found in 12 of the 21 biopsies showing chronic rejection. Isolated CsA nephropathy was associated with the best outcome. The decrease in sCt was, respectively, 37%, 14% and 7% in patients with CsA nephrotoxicity, grade 1 chronic rejection and grade 2 chronic rejection ($P = 0.01$). Renal function significantly improved in patients with grade 1 chronic rejection and remained stable in patients with grade 2 chronic rejection (Table 3). Nevertheless, the proportion of patients in whom renal function improved was similar in Banff 1 and 2 chronic rejection (75% vs. 66%; $P = 0.19$). Moreover, variation in sCt were significantly different in each histological group before and after conversion, indicating an improvement in GFR or a decrease in the steepness of the slope of worsening (Table 4). No histological elementary lesion was predictive of outcome. A follow-up biopsy was available in 24 patients (Banff 1 chronic rejection, ten; Banff 2 chronic rejection, seven; isolated CsA nephropathy, seven). Mean interval between the two biopsies was 14 ± 2 months. Seven patients were not biopsied during follow-up (refusal, four; death, three). Worsening shown by histology was found in four patients, whereas, histologically shown lesions were stable in 15 and improved in three. Improvement of histologically shown lesions was observed

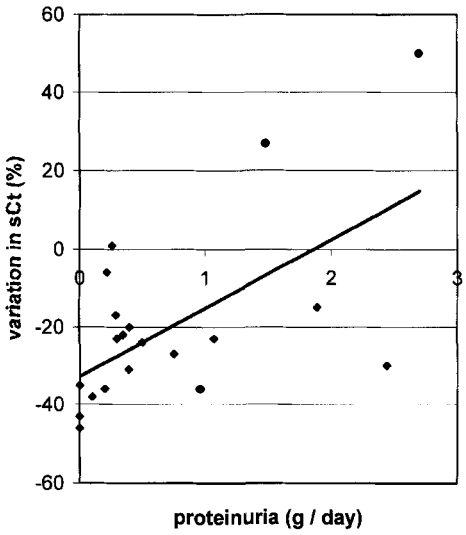

Fig. 1. Relationship between proteinuria at baseline and variation in renal function after conversion

Table 3. Evolution of renal function according to histological diagnosis

Parameter	Before conversion	Last evaluation	*P*
CsA nephropathy ($n = 10$)	213 ± 36	134 ± 49	< 0.01
CR grade 1 ($n = 12$)	214 ± 45	185 ± 47	0.02
CR grade 2 ($n = 9$)	259 ± 50	240 ± 67	0.16

Table 4. Variations in sCt before and after conversion according to histological diagnosis

Characteristic	Before conversion	Last evaluation	*P*
CsA nephropathy ($n = 10$)	+11%	–37%	< 0.01
CR grade 1 ($n = 12$)	+16%	–13%	0.01
CR grade 2 ($n = 9$)	+34%	–7%	< 0.01

only in patients with CsA nephrotoxicity. There was no relationship between histological changes and variations in renal function.

Proteinuria

At the end of follow-up, there was a slight but significant increase in proteinuria (0.79 ± 0.6 vs. 1.79 ± 1.08 g/day; $P = 0.04$). The variation in proteinuria was not related to baseline proteinuria ($r = -0.07$; $P = 0.31$).

Acute rejection

Two patients (6.5%) experienced late acute rejection, respectively 13 and 24 months after CsA withdrawal. In one case, non-compliance was admitted by the patient who had spontaneously reduced MMF dosage to 500 mg a day. The two rejections were treated by high-dose steroids and tacrolimus. Renal function improved in one case, returning to pre-rejection levels of creatinine, and remained stable in the other case.

Safety and tolerability

Nine patients (29%) experienced transient diarrhoea and abdominal pain at the beginning of MMF therapy. MMF was stopped in one patient because of intractable diarrhoea. Diarrhoea rapidly improved after MMF withdrawal.

Nine patients (29%) experienced ten systemic infections (pneumonia, five; pyelonephritis, one; bacteriaemia, three; systemic toxoplasmosis, one) requiring hospitalization. This corresponds to 12 serious infections per 1,000 patient-months. By contrast, only one patient had been hospitalized during the year before conversion (infection rate = 2.6/1,000 patient-months; $P < 0.01$).

Even when mean Hb concentration remained stable during follow-up, six patients experienced severe anaemia during MMF therapy. Anaemia slightly improved after MMF dosage reduction. In two patients, EPO was successfully administered to correct anaemia. CD4 cell count significantly decreased after conversion ($500 \pm 168/mm^3$ vs. $389 \pm 157/mm^3$; $P = 0.005$). The variation in Hb concentration was related to the changes in CD4 cell count ($r = 0.31$; $P = 0.01$).

Cancers occurred in three patients (13%) (lung, one; breast, one; rectum, one). MMF was withdrawn in all of them. One patient died from severe infections following anti-neoplastic chemotherapy. One was returned to dialysis 1 year after the withdrawal of MMF and is still in remission 2 years after surgery and radiotherapy for breast cancer.

Cardiovascular profile

Mean systolic and diastolic blood pressure did not vary during follow-up. However, the mean number of antihypertensive drugs being used significantly decreased (1.83 ± 1.1 vs. 0.9 ± 0.5; $P < 0.01$).

Serum LDL-cholesterol and triglyceride levels were 1.38 ± 0.5 g/l and 1.72 ± 0.4 g/l, respectively, before conversion and 1.24 ± 0.3 g/l ($P = 0.03$) and 1.39 ± 0.3 g/l ($P = 0.01$) at the end of follow-up. Ten patients received statins before conversion. Drug dosage was not modified in any case.

Discussion

Our study shows that CsA withdrawal with a concomitant switch from AZA to MMF allows a significant and prolonged improvement in renal function in patients with chronic allograft nephropathy. This result confirms, in a larger cohort followed for a longer duration, the results of our preliminary report [6].

Because the addition of MMF to standard CsA doses did not have any significant benefit in patients with deteriorating graft function due to chronic rejection [18, 19], CsA withdrawal, rather than MMF addition, may be the critical factor in improving graft dysfunction. In almost all the patients, renal function improved remarkably quickly after CsA withdrawal and remained stable thereafter, suggesting that the improvement in renal function is mainly due to reversal of the vasoconstrictor effects of CsA. This result is in accordance with previous reports demonstrating an increase in renal plasma flow after CsA dose reduction in renal transplant recipients with chronic allograft nephropathy [11]. Whether CsA-associated morphological changes or other features of chronic allograft nephropathy are reversible would be assessed through controlled studies with repeated biopsies.

Both hypertension and dyslipidaemia have been implicated in the pathogenesis of chronic allograft dysfunction. Modena et al. showed that the rate of decline in renal function, as estimated by serum creatinine, was correlated with the level of diastolic blood pressure [15]. This result was in agreement with the findings of Cheigh et al. [4] that hypertension is associated with a greater rate of graft loss. There is also a great concern about the influence of dyslipidaemia on chronic allograft nephropathy. Recently, Massy et al. [14] have shown that hypertriglyceridaemia was a risk factor for chronic allograft rejection. In this study, we noted an improvement in both blood pressure control and in lipid profile after CsA withdrawal. These effects may have a favourable influence on graft survival.

We observed a strong inverse relationship between proteinuria at baseline and the degree of improvement in

renal function after CsA withdrawal and conversion from AZA to MMF. Several explanations can be put forward. First, transplant recipients with declining creatinine clearance tend to have a higher prevalence of proteinuria [12]. First et al. [8] reported that more than three-quarters of patients with greater than 2 g/day of proteinuria lost their graft, the majority within a year of developing this degree of proteinuria. Thus, it is conceivable that in proteinuric chronic allograft nephropathy, the improvement of renal haemodynamic conditions is counterbalanced by the more severe natural course of the renal disease. Of even greater importance is the association of even 1 g/day of proteinuria with the degree of histological evidence of chronic rejection, especially vascular thickening, interstitial fibrosis, glomerular basement-membrane reduplication, and a composite index of chronic rejection [13]. These features are also more likely to be associated with immunologically mediated damages rather than with CsA nephrotoxicity. This hypothesis is confirmed by the observation that renal function improved more in patients with isolated CsA nephropathy. However, this result must be mitigated by the fact that even when renal function did not improve in patients with more-severe histological lesions, the decline in renal function was stopped after CsA withdrawal and conversion from AZA to MMF.

We observed an increase in proteinuria after CsA withdrawal. Because we did not observe marked changes in histology, and because renal function remained stable even several months after the increase in proteinuria, worsening of chronic rejection after CsA withdrawal is unlikely. CsA has non-specific anti-proteinuric properties. It is possible that the haemodynamic changes induced by CsA withdrawal explain the increase in proteinuria.

Nine patients experienced systemic infections requiring hospitalization during the study period. It is likely that MMF at full dose is more potent as an immunosuppressive therapy than low-dose CsA plus low-dose AZA. Reduced doses are probably required in patients with renal failure, and monitoring MMF pharmacokinetics could be useful in this situation. We observed a significant decrease in CD4 cell count after conversion. Our group has previously reported the predictive value of CD4 lymphocytopenia as a marker of over-immunosuppression in RTR [6]. The decrease in CD4 cell count may at least partly explain the increased incidence of infections after conversion.

To conclude, CsA withdrawal with a concomitant switch from AZA to MMF allows a substantial and durable improvement in renal function. Both allograft histology and proteinuria at baseline are predictive of the evolution of renal function after conversion. Despite this beneficial effect, physicians should consider the risk of over-immunosuppression possibly associated with this therapeutic strategy.

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