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Long-term efficacy and safety of mycophenolate mofetil in liver transplant recipients with calcineurin inhibitor-induced renal dysfunction

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Abstract Long-term survival after orthotopic liver transplantation (OLT) is mainly influenced by adverse events caused by immunosuppression. Several studies have shown the efficacy of mycophenolate mofetil (MMF) in improving calcineurin inhibitor (CI)-induced nephrotoxicity with concomitant reduction or withdrawal of CI. In this prospective study we assessed the long-term effect and safety of MMF. Thirty-two OLT recipients with significant renal impairment due to either cyclosporine A ($n=25$) or tacrolimus ($n=7$) were enrolled in this study. CIs were reduced stepwise by at least 70%. Mean serum creatinine had decreased from 2.63 ± 0.39 to 1.74 ± 0.34 mg/dl after 1 month, and this improvement was maintained within a follow-up period of 4.8 ± 0.6 (range 3.1–6.0) years, without major immunological or non-immunological side effects. Of all participants, 88% showed a significant reduction, and 41% even a normalization, in their serum creatinine level. In addition, MMF conversion, within 6 months of OLT, appears to be crucial in order to improve or even normalize renal function. This study demonstrates the long-term efficacy and safety of MMF in OLT recipients with CI-induced nephropathy.

Keywords Mycophenolate mofetil · Nephrotoxicity · Immunosuppression · Calcineurin inhibitor

Introduction

Orthotopic liver transplantation (OLT) has become a well-established, routine treatment option for end-stage liver diseases [1]. The success of OLT has improved dramatically over the past few decades [2]. An important aspect of this success is the availability of more potent immunosuppressive drugs. In particular, the calcineurin inhibitors (CIs) cyclosporine A (CsA) and tacrolimus (FK) have been milestones in the improvement of post-transplantation survival. Both CIs, which are T cell-specific immunosuppressants, are excellent agents for preventing rejection after OLT [3]. On the other hand, both drugs can cause multiple non-immunological adverse events, such as renal dysfunction, neurological disorder, arterial hypertension and metabolic disorders. This limits their use in some patients and can be a major cause of morbidity and mortality [4, 5].

This dilemma between excellent long-term transplant survival and serious side effects has unleashed efforts to develop newer immunosuppressive agents. One of these agents is mycophenolate mofetil (MMF), a reversible inhibitor of inosine monophosphate dehydrogenase [6]. This effect inhibits de novo purine synthesis and prompts the relatively selective inhibition of B and T lymphocyte proliferation. The non-immunological adverse-event

profile of this drug is different from that of CIs, as there is no reported nephrotoxicity and neurotoxicity. Several studies have demonstrated the effectiveness of MMF in the treatment of acute rejection [7, 8, 9]. Various trials have shown that replacement of CIs with MMF in patients who have undergone OLT can improve renal function [10, 11, 12]. To date, however, no study has addressed the long-term outcome of patients with conversion to MMF because of impaired renal function due to CIs. Therefore, this prospective study was conducted to determine the long-term efficacy and safety of OLT recipients with CI-associated renal dysfunction.

Patients and methods

Patients' demographics

Between February 1996 and January 1999, 32 liver transplant patients (22 male/ten female) with stable liver function under CI-based immunosuppression were enrolled in this prospective study. All patients gave their informed consent, and the study was approved by the local ethics committee. All participants presented with a slowly progressing impaired renal function as a side effect of CIs. Renal dysfunction was defined as a serum creatinine level higher than 1.4 mg/dl and a blood urea nitrogen (BUN) level higher than 50 mg/dl measured on two different occasions at least 1 month apart. Primary causes of renal dysfunction, including hepato-renal syndrome prior to OLT, were excluded. All patients presented with normal parameters of renal function before OLT. Patients with a history of severe rejection episodes were also not considered for this study. Stable graft function was defined as normal or slightly elevated levels of aminotransferases and bilirubin due to non-immunological reasons (e.g. recurrent viral hepatitis, bile flow impairment). Mean age was 57 ± 8 (range 33–74) years. Underlying liver diseases were: viral hepatitis ($n = 11$); cryptogenetic cirrhosis ($n = 10$); haemochromatosis ($n = 4$); autoimmune hepatitis ($n = 3$); cholestatic liver disease ($n = 2$); cholangiocellular carcinoma ($n = 2$). In six patients hepatocellular carcinoma was diagnosed prior to OLT. Demographic data are summarized in Table 1.

Pre-MMF immunosuppression

A triple immunosuppressive therapy regimen, either CsA or FK, in combination with corticosteroids (tapered stepwise within 3 months) and azathioprine is generally used at our centre. In this study 25 patients received CsA-based immunosuppression with a target

Table 1 Baseline data of patients

Parameter	Total	Early switch (< 6 months)	Late switch (> 6 months)
Number	32	14	18
Age in years (mean ± SD)	57 (range 33–74)	60 (range 50–74)	54 (range 33–64)
Gender (male/female)	22/10	13/1	9/9
Underlying liver disease			
Viral hepatitis	11	9	2
Cryptogenetic liver disease	10	3	7
Haemochromatosis	4	0	4
Autoimmune hepatitis	3	1	2
Cholangiocellular carcinoma	2	1	1
Cholestatic liver disease	2	0	2
Immunosuppression at study entry			
CsA	25	10	15
FK	7	4	3
Corticosteroids	18	12	6
Azathioprine	14	5	9
CsA trough level (ng/ml)	151 (range 64–253)	171 (range 64–253)	140 (range 97–250)
FK trough level (ng/ml)	10,6 (range 6.9–13.6)	12.2 (range 13.6–10.5)	9.3 (range 6.9–12.1)
Immunosuppressant at end of study			
CsA	18	7	11
FK	5	3	2
Corticosteroids	11	6	5
MMF	29	13	16
CsA trough level (ng/ml)	32 (range 0–67)	27 (range 0–67)	33 (range 0–65)
FK trough level (ng/ml)	2.7 (range 1.5–5.8)	3.4 (range 1.5–5.8)	1.7 (range 1.5–1.8)
Mean time of MMF conversion following OLT (months)	25.6 (range 1.2–127.8)	1.9 (range 1.2–4.2)	43.9 (range 9.1–127.8)
Mean follow-up period after MMF initiation (months)	57.1 (range 37.1–72.5)	55.0 (range 37.1–66.5)	59.1 (range 46.1–72.5)

trough level of 150–200 ng/dl within the first 3 months and approximately 100 ng/dl later. FK was the primary immunosuppressive agent in seven patients (levels between 6.9–13.6 ng/dl). Additionally, 18 patients were treated with corticosteroids and 14 patients with azathioprine. (Table 1)

MMF conversion

Conversion to MMF was started 25.6 ± 34.7 (range1.2–127.8) months after OLT. In all patients MMF was initiated at an oral dose of 1,000 mg twice daily. At the same time azathioprine was discontinued. After the patients had been receiving the full dose of MMF for 3 days, the CIs were gradually reduced to the lowest possible level (at least 70%), as determined by normal levels of transaminases. In nine patients the CIs were completely discontinued. Corticosteroids were tapered stepwise ($n = 18$) and, if possible, completely withdrawn ($n = 7$).

Follow-up

Renal function was assessed by the monitoring of the serum creatinine and blood urea nitrogen levels at least every 3 months. In approximately one-third of patients, creatinine clearance was performed. Transaminases, γ-glutamyl-transpeptidase, alkaline phosphatase and bilirubin values were obtained at each follow-up visit at least twice a year. After MMF initiation, liver biopsies were performed whenever graft dysfunction was suspected, as determined by elevated liver function parameters. Rejection episodes were treated with corticosteroid bolus therapy or increasing CI doses. The mean follow-up time of all patients after MMF initiation was 57 ± 7.5 (range 37–72) months.

Adverse reactions

Adverse reactions were recorded prospectively, based on the patients' reports. Most adverse reactions were based on patient's complaints, such as nausea, vomiting, diarrhoea and abdominal discomfort. Leukopenia was defined as a white cell count $\leq 3{,}000/mm^3$. Thrombocytopenia was defined as a platelet count $< 100{,}000/mm^3$.

Statistical analysis

Data are expressed as mean ± standard deviation. Student's t-test was used to determine the significance of any difference in means. A difference was considered statistically significant for a P value of < 0.05.

Results

Renal function

Between February 1996 and January 1999, 32 patients presented with stable liver function but impaired renal function and were eligible for this study. Conversion to MMF was, on average, 25.6 (range 1.2–127.8) months after OLT. The mean follow-up period for all patients after commencement of MMF treatment was 4.8 ± 0.6 (range 3.1–6.0) years. The overall serum creatinine level was 2.63 ± 0.39 (range 1.96–6.62) mg/dl, and the initial urea level was 116.2 ± 40.7 (range 61.5–232.3) mg/dl at the beginning of this study. One month after the patients' conversion to MMF, a significant improvement in renal function had already been detected: the creatinine value had decreased to 1.74 ± 0.34 (range 1.15–2.8) mg/dl and blood urea nitrogen had decreased to 79.5 ± 45.6 (range: 20.0–231.4) mg/dl. Creatinine clearance was available in one-third of patients, and these values were in good correlation with the serum creatinine levels. At the last follow-up, 28 patients (88%) still displayed the same stable improvement in their renal parameters (Fig. 1A). There was no difference in this significant improvement in serum creatinine levels between patients who were converted within 6 months ("early switch group") and those converted later ("late switch group") (Fig. 1B, C). In total, 13 patients (41%) had normal creatinine values at the end of the study. However, as depicted in Fig. 2, early conversion to MMF seems to lead to a higher percentage of normalization of renal function parameters. Nine patients (64%), who were converted to MMF within 6 months of OLT, showed normalization of creatinine levels, but only four patients (22%) in the late switch group did. In four patients, however, improvement of renal function could not be achieved, despite marked reduction of CIs. Three of those patients developed chronic renal failure and needed continuous haemodialysis. One patient showed histological evidence of minimal-change nephritis, and another patient had clinical characteristics of diabetic nephropathy as a potential (co)factor to CI-induced renal failure. Two patients are still on continuous haemodialysis and are currently awaiting a kidney transplantation. One patient died due to cardiovascular problems.

Non-immunological adverse events

The majority of adverse reactions were gastrointestinal (25%) or myelosuppressive (28.1%). The most common gastrointestinal adverse reactions were: diarrhoea (25%); abdominal pain (3.1%); and nausea and vomiting (6.3%). All gastrointestinal reactions,

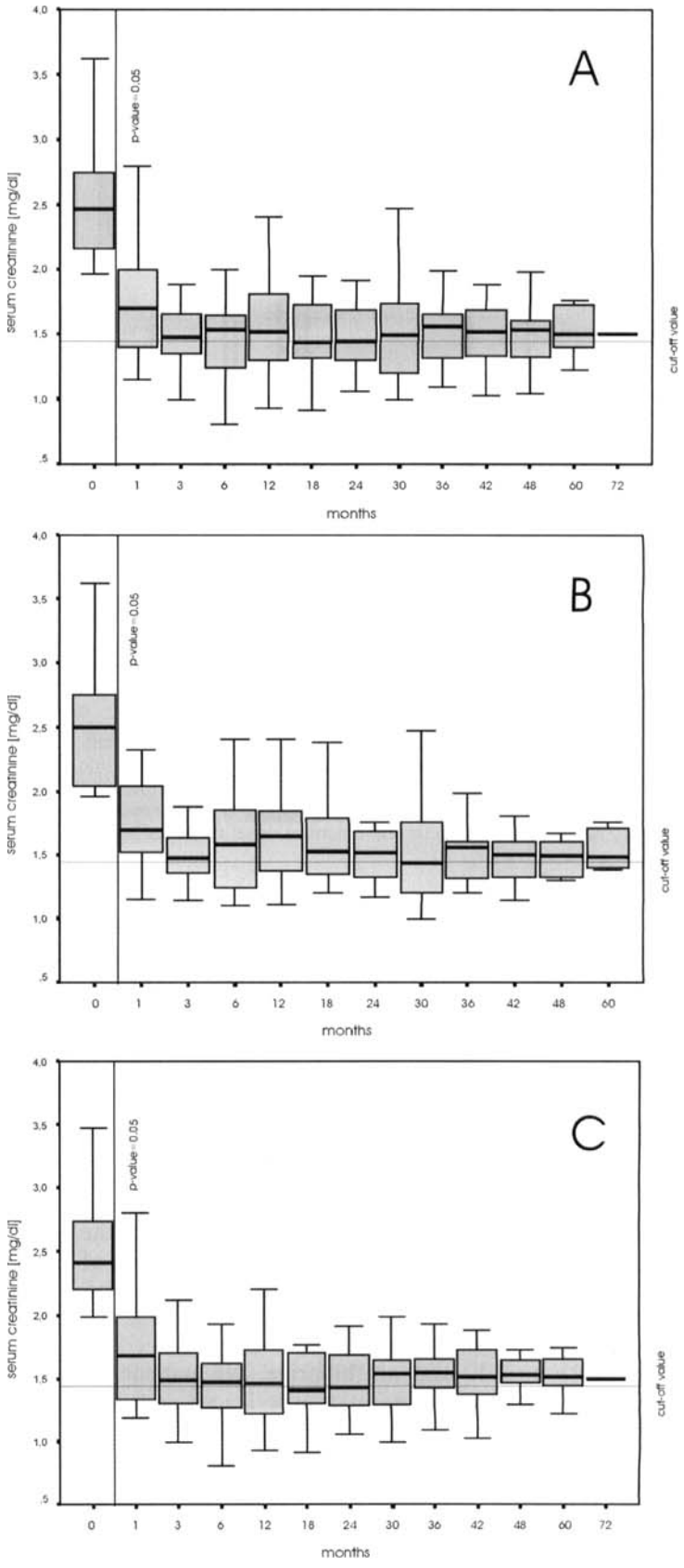

Fig. 1A–C Mean serum creatinine values before and after MMF conversion. **A** total; **B** early switch group; **C** late switch group

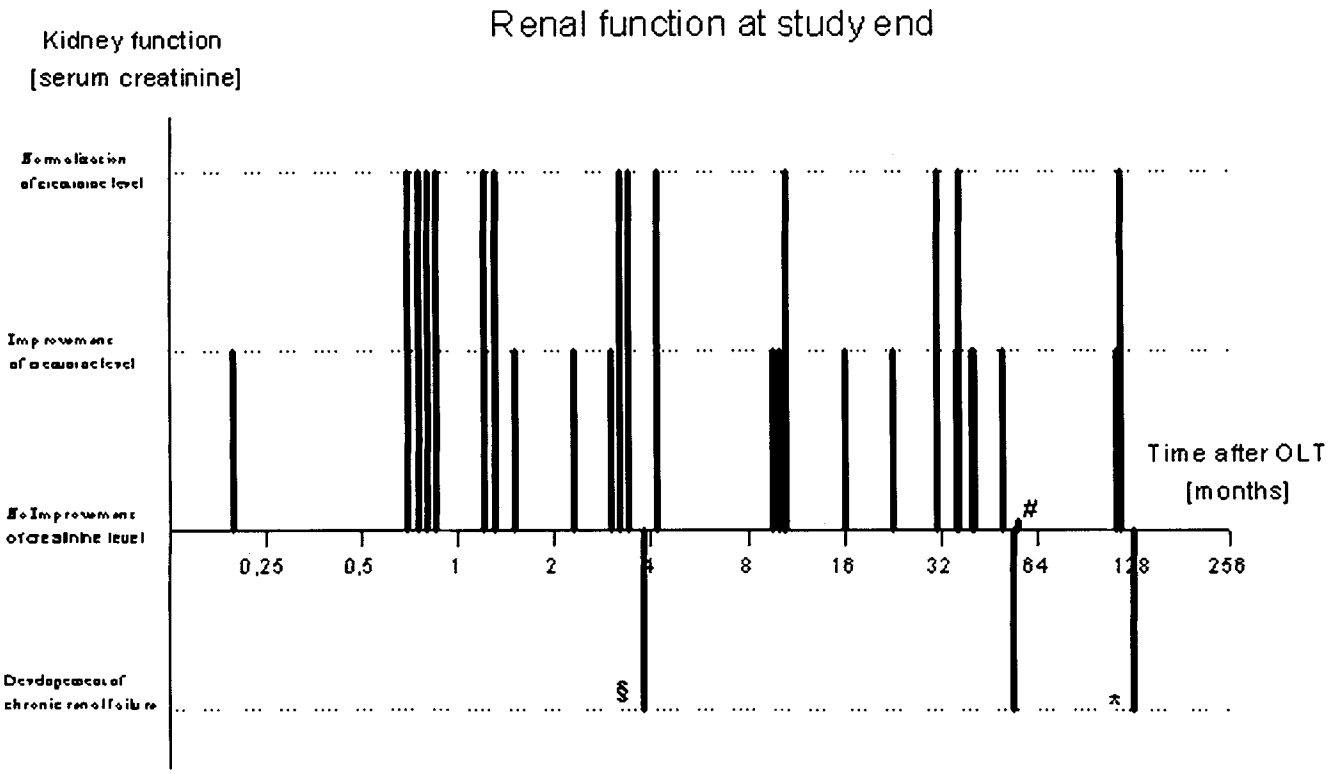

Fig. 2 Renal function at the end of the study: normalized, improved or deteriorated renal function with regard to the time interval of MMF conversion following OLT (* showed histological evidence of minimal-change nephritis, § clinical characteristics of diabetic nephropathy, # no improvement in renal function

however, were reversible in the majority of patients without any dose reduction. Other adverse reactions probably due to MMF were as follows: leukopenia (18.8%); thrombocytopenia (9.4%); fatigue (6.3%); headache (3.1%); hair loss (3.1%).

Immunological adverse events

Only two (6%) acute rejection episodes occurred within the follow-up period. One patient experienced biopsy-confirmed acute rejection 14 months after entry into the study. The rejection episode responded to resumption of CsA. The second episode of acute rejection occurred 9 months after the patient's entry into the study. The patient regained normal liver function after conversion from MMF to FK and azathioprine. No patient required antilymphocyte therapy or experienced graft loss due to rejection. Other causes of elevated liver parameters were: recurrence of viral hepatitis ($n=4$) cholangiocellular carcinoma ($n=1$) cholestatic liver disease ($n=1$) and autoimmune hepatitis ($n=1$). Four patients (13%) developed an anastomotic stricture. CMV disease was seen in three patients (9.4%), and all three required specific treatment.

Death and discontinuation of therapy

Five patients (16%) died 20, 40, 52, 55 and 64 months, respectively, after conversion or 21, 44, 56, 59 and 105 months, respectively, after OLT. Two died because of cardiovascular problems, one due to de novo pancreatic cancer, one because of recurrent cholangiocellular carcinoma and one because of sepsis due to cholangitis.

Discontinuation of therapy was necessary in three patients (9.4%). One patient was taken off MMF 17 months after conversion because of severe diarrhoea, the second due to marked hair loss, and, in the third patient, MMF was stopped secondary to a moderate acute rejection episode.

At the end of the study 18 patients (56%) received low-dose CsA (mean trough levels of 32 ng/dl; range below the detection level–67 ng/dl) and five (16%) were still on low-dose FK (mean trough levels of 2.7 ng/dl; range 1.5–5.8 ng/dl). CIs were completely withdrawn in nine patients (28%), who afterwards received a combination of MMF (2,000 mg daily) and prednisolone (5–10 mg daily). Only in patients with minimal ($n=2$) or discontinued ($n=9$) doses of CIs were steroids continued, at a dose of 5 mg

prednisolone. In the other patients steroids were tapered within 3 months.

Discussion

The calcineurin inhibitors CsA and FK currently serve as the basis for almost every immunosuppressive regimen in liver transplant recipients. The introduction of CsA and FK prompted a dramatic improvement in survival after OLT. Both CIs, however, are often associated with side effects, most commonly nephrotoxicity, which leads to progressive impairment of renal function [4, 5]. Several trials have recently shown the short-term effect of conversion to MMF, which does not exhibit nephrotoxic properties, concomitant with a reduction in or withdrawal of CIs in order to improve renal function [5, 11, 12]. The purpose of this study was to assess the long-term efficacy and safety of MMF in OLT patients with CI-induced renal dysfunction.

This paper shows that kidney function, as assessed by serum creatinine concentrations, significantly improved in the majority (88%) of patients with CI-induced nephrotoxicity (Fig. 1). Thirteen patients (41%) even achieved complete normalization of renal function parameters. Unfortunately, three patients developed progressive renal impairment followed by chronic renal failure, despite CI withdrawal and conversion to MMF. Two of those patients, however, had potential additional risk factors for chronic renal insufficiency. One had minimal-change nephritis on kidney biopsy, and one exhibited clinical evidence of diabetic nephropathy due to a long history of poorly controlled insulin-dependent diabetes mellitus. This finding indicates that, especially in patients with concomitant risk factors for renal disease, such as diabetes, CIs should be reduced or withdrawn as soon as possible in order to avoid renal failure.

The third patient was converted to MMF very late (128 months) after OLT, when he had already shown markedly elevated renal function parameters for an extended period of time. Reintroduction of CsA (at his own request, because of hair loss most probably due to MMF), although at a very low dose (25 mg q.d.), caused further deterioration in kidney function, which, consequently, required haemodialysis.

The exact mechanism of CI-induced nephrotoxicity remains poorly understood and its pathophysiology incompletely defined. Acute reversible renal failure appears to be mediated by a reduction in kidney perfusion caused by an increase in vascular resistance and a concomitant reduction in glomerular filtration rate [13]. In contrast, chronic nephrotoxicity is associated with structural changes in the kidney and thus represents an almost irreversible or even progressive type of tissue damage [14, 15]. Additionally, it has been shown that late-onset CI-induced renal failure in OLT recipients is associated with an extremely high mortality rate [5].

Interestingly, in our study, two-thirds of patients, who were converted within 6 months of OLT, attained normal creatinine values; in contrast to only one-fifth of the remaining patients (Fig. 2). This finding is in accordance with previous reports that have suggested that CIs should be discontinued early and, ideally, before any severe or irreversible damage occurs to the kidney [10, 11, 12].

A serious drawback to dose reduction or withdrawal of CIs is reduced immunological safety. Severe acute or chronic rejection episodes have been reported after CsA withdrawal, although these patients received azathioprine, which seems to be less potent than MMF [16, 17]. In our study two patients (6%) developed a biopsy-proven acute cellular rejection episode. One occurred 14 months after MMF conversion, when CsA was completely discontinued. However, the patient regained normal liver function after reintroduction of CsA at subtherapeutic levels. Fortunately, his kidney function did not deteriorate again. In the second case, the rejection episode was diagnosed 9 months after the patient's enrolment into this study, when he was on low-dose steroids and MMF. As the rejection was moderate, he was converted to low-dose FK, prednisolone and azathioprine, after steroid bolus therapy; his liver function parameters normalized thereafter.

None of our patients received MMF monotherapy for reasons of immunological safety, especially not in the early period after OLT. In fact, a recently published study showed that the immunological risk is increased when MMF is administered alone. In that study no rejection episode occurred in patients on low-dose steroids and MMF, whereas several patients on MMF monotherapy had rejections [12]. Herrero et al. also noted rejection episodes in steroid-free patients after CsA replacement with MMF [10]. Only in some selected patients did MMF monotherapy seem to be safe and allow a significant improvement in renal function [11, 18].

Despite the beneficial effects, there is also evidence of potentially adverse effects due to MMF. It is known that MMF can cause several dose-limiting gastrointestinal and bone marrow suppressive adverse events. Indeed, half of our patients experienced side effects, in particular gastrointestinal discomfort (nausea, diarrhoea), leukocytopenia and thrombocytopenia. However, almost all adverse events were reversible. In only one patient was MMF discontinued due to severe diarrhoea, which ceased afterwards.

In conclusion, MMF conversion is a safe therapeutic option with minimal immunological risks for patients with CI-induced nephrotoxicity. Most importantly, our long-term study shows that MMF conversion is highly efficacious and significantly improves renal function in the majority of patients.

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