Mycophenolate mofetil in kidney transplantation

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Mycofenolaat mofetil bij niertransplantatie

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Chapter 1

INTRODUCTION

MYCOPHENOLATE MOFETIL, CELLCEPT®

a new immunosuppressive drug with great potential in internal medicine

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Abstract

Mycophenolate mofetil is a new immunosupressive drug, exhibiting its effect through inhibition of proliferation of T- and B-lymphocytes. Superior efficacy of mycophenolate mofetil compared to azathioprine, in combination with cyclosporine and prednisone, in the prevention of acute rejection in organ transplantation has made mycophenolate mofetil one of the standard immunosupressive drugs after transplantation. Mycophenolate mofetil also is an interesting candidate drug for many other, mainly auto-immune mediated diseases. The use of mycophenolate mofetil in several of these diseases is discussed. The definitive place of mycophenolate mofetil will depend on the results of randomised trials currently under way.

1. Introduction

After the introduction of mycophenolate mofetil (MMF) as part of maintenance immunosuppressive therapy in organ transplantation several new indications, mostly auto-immune diseases involving T- and B-lymphocytes, are under investigation for this drug. Initially MMF was used in selected cases, that were found to be resistant to standard treatment. After demonstrating efficacy of MMF in these cases, MMF was used less restrictively in larger numbers of patients. It is possible that MMF will replace azathioprine and methotrexate as treatment of choice for many indications for which both drugs are currently employed. Because we expect an increased usage of MMF in patients suffering from a wide variety of auto-immune diseases, we present an overview of pharmacological characteristics and clinical use of MMF in internal medicine.

2. Historical development

Xenobiotic immunosuppressive agents are molecules produced by micro-organisms, with or without synthetical modification. They are dissimilar from naturally occurring mammalian molecules. Mycophenolate mofetil is an example of this class of agents. The development of MMF was based on a postulate made by Allison and colleagues, that proliferation of activated T and B lymphocytes, unlike most other cells, preferentially relies on the *de novo* purine synthesis, with a minor/insignificant contribution of the salvage pathway for purine synthesis. This selective inhibition of lymphocyte proliferation was illustrated in two clinical

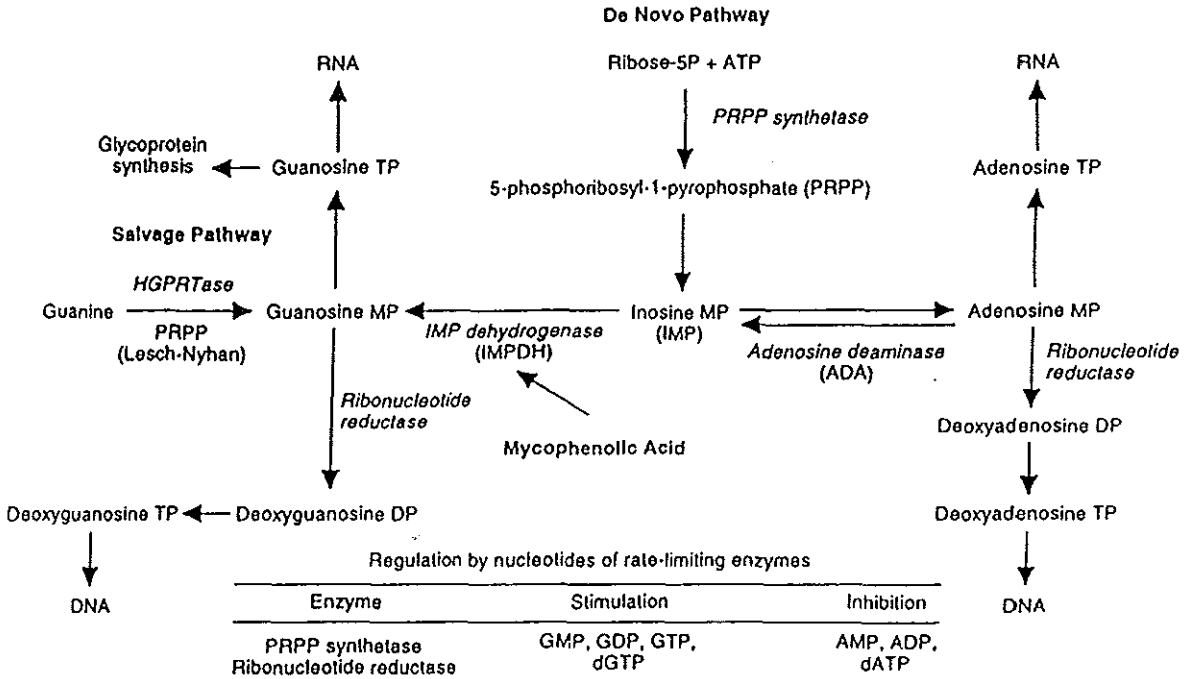

Fig. 1. The purine synthesis pathways and the inhibition of mycophenolic acid on the rate-limiting step (enzyme) of the de novo pathway (adapted with permission from Allison and Eugui [18]).

syndromes. In children with an inherited immunodeficiency, adenosine deaminase deficiency, a marked depletion of functional T and B lymphocytes exists. In contrast, children with Lesch-Nyhan syndrome, characterised by a deficiency in the salvage pathway of purine synthesis (lack of hypoxanthine-guanine phosphoribosyltransferase), nearly normal levels of functional T and B lymphocytes are present (1,2). This observation led to the production of MMF, which was formed by esterification of mycophenolic acid, allowing a more reliable absorption from the gastro-intestinal tract. Mycophenolic acid was first described as early as 1896 and was "rediscovered" as a compound with anti-cancer activity but also an anti-proliferative effect in the 1960's (3-5). Mycophenolate mofetil is a non-nucleoside, non-competitive, reversible inhibitor of inosine 5'-monophosphate dehydrogenase (IMPDH), one of the crucial enzymes in the *de novo* pathway of purine synthesis (figure 1) (6).

3. Pharmacology

The chemical name for MMF is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate, which has an empirical formula of $C_{23}H_{31}NO_7$ (figure 2), with a molecular weight of 433.5 Da.

Mycophenolate mofetil is a pro-drug (morpholinoethyl ester) for mycophenolic acid (MPA), a fermentation product of several *Penicillium* species. Mycophenolic acid is a non-nucleoside, non-competitive, reversible inhibitor of inosine 5'-monophosphate dehydrogenase (IMPDH). This is the rate-limiting enzyme in the *de novo* synthesis of guanosine triphosphate (GTP). Through this property, MPA decreases the intracellular guanine nucleotide pools (4,7-9). The addition of exogenous guanine, guanosine or deoxyguanosine restores intracellular GTP and dGTP pools and reverses the antiproliferative effect of MMF (10-12). Thus far 2 isoforms of human IMPDH have been identified and sequenced, named type I and type II(13). Type I IMPDH is constitutively expressed and is the predominant isoform in normal resting cells, whilst type II is selectively upregulated in neoplastic and replicating cells. Griesmacher et al demonstrated inhibition of both isoforms of IMPDH *in vitro* by MPA, but also by MPA-glucuronide (MPAG)(14).

The depletion of GTP pools in lymphocytes and monocytes by MMF inhibites glycosylation of fucose and mannose, which leads to less stabile membrane glycoproteins needed to bind adhesion molecules (selectins). These adhesion molecules are in turn required for the recruitment of leucocytes at sites of inflammation or graft rejection(15-19). The additional effects of MMF, which are separate from the anti-proliferative effect but do contribute to its

overall immunosuppressive potential, explain why MMF is so much more effective in the prevention of for example acute allograft rejection than other anti-proliferative drugs, such as azathioprine.

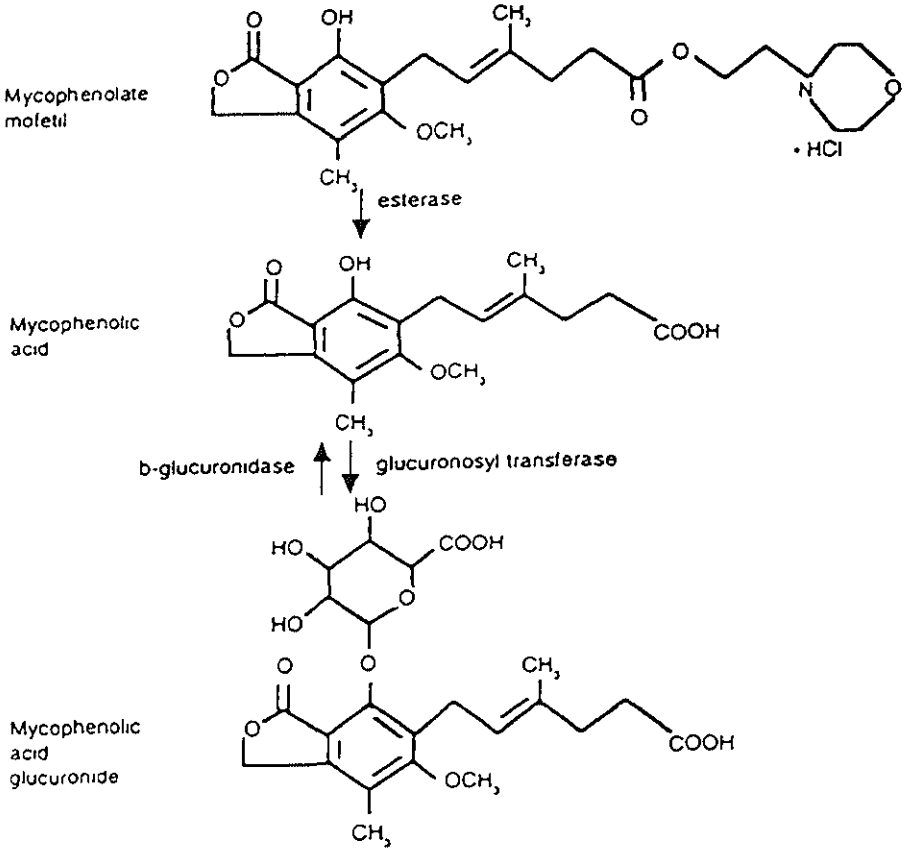

Fig. 2. The structural chemistry formula of mycophenolate mofetil, mycophenolic acid and mycophenolic acid glucuronide (adapted with permission from Allison and Eugui [18]).

Furthermore, antibody formation is also inhibited by MMF, in a dose-dependent manner. This was demonstrated in a rat model with nearly abolished antibody formation against xenogeneic cells (9,20), and also in mice with strongly diminished IgG formation in response to influenza virus hemagglutinin(21). Other studies also demonstrated an inhibition of antigen-induced B-

lymphocyte antibody formation(10,22-24). A possible positive role of MMF in the prevention of chronic rejection after experimental organ transplantation has been suggested based on results of *in vivo* studies, which showed reduced smooth muscle and endothelial cell proliferation (25-28). After oral ingestion MMF is rapidly and almost completely absorbed and hydrolysed to MPA (90% bioavailability), which is conjugated in the liver to MPAG. A substantial amount of enterohepatic re-circulation exists for MPAG, with a decrease of 40% of MPA area-under-the-curve (AUC) when concomitantly administered with cholestyramine in healthy volunteers (29). Mycophenolic acid and MPAG are, respectively, 97% and 82% bound to albumin. Both in vitro and in vivo studies demonstrate that only the free fraction (non-protein bound) of MPA is avaliable to inhibit IMPDH(30-32) (33,34). The mean terminal half-life of MPA is 15.8 h, including enterohepatic re-circulation. Excretion of MPAG is predominantly through the kidneys (94%) and a small amount is eliminated in the faeces (6%) (35). Both MPA and MPAG can be measured in blood by HPLC or by immunoassay (MPA). Only MMF is available for oral use, in capsules of 250 mg and tablets of 500 mg, and intravenous use (mycophenolate mofetil hydrochloride). The inhibition of IMPDH in lymphocytes can be measured, albeit technically difficult, and might be a tool for pharmacodynamic monitoring of MMF although currently its use is predominantly in research settings (36,37).

4. Drug interactions

Four potential mechanisms for drugs to interact with MMF involve: interference with absorption, the enterohepatic re-circulation, renal tubular excretion (competition between MPAG), or competitive interference of co-administered drugs which are albumin bound. Co-administration of antacids reduces both mean plasma MPA-AUC_{24} and C_{max} for MPA, which is consistent with a reduced absorption (38). Examples of the drug interactions with enterohepatic re-circulation are concomitant use of cholestyramine and other bile acid sequestrants, or of antibiotics directed against gut flora which contain glucuronidase activity (29). It has been suggested that tacrolimus increases MPA trough levels with concomitant use of MMF and tacrolimus in renal transplant recipients compared to patients using CsA and MMF (39,40). However, we demonstrated that it is in fact CsA which decreases MPA trough levels with concomitant use in a cross-sectional study and in a longitudinal study(41,42). This effect of CsA is also probably related to an interaction involving the enterohepatic re-circulation, as suggested by our clinical studies (42,43), and by an experimental study (44).

Plasma MPA levels are not affected when a renal tubular excretion interaction occurs, although clearly MPAG levels are raised. Thus far no clinical consequences related to increased MPAG levels have been reported, although it is conceivable that raised plasma concentrations of the competing drug do cause side-effects. In renal transplant patients with delayed graft function the AUC of MPAG is 2 to 3 times higher than in patients without delayed graft function and there is also an increase in the free fraction of AUC_{0-12} MPA (45,46). In patients with chronic renal insufficiency a decreased protein binding exists for MPA, which in turn results in an increase in free MPA concentrations(31,47). It is uncertain what the explanation of this impaired albumin binding is. Thus far no known drug combinations exist for which MMF is contraindicated.

5. Drug related adverse events

The overall tolerability of MMF partly resembles that of azathioprine. Specific MMF related side-effects are predominantly of a gastro-intestinal nature, and include diarrhoea, abdominal pain, nausea and vomiting. These side-effects appear dose related, possibly MPA level related (48) (49) (50) (41), and respond quickly to dose reduction. Adverse events generally occur shortly after the initiation of MMF therapy. Haematological side-effects are also relatively frequent, with leucopenia most commonly reported in a frequency similar to azathioprine(48) (49). Despite its postulated specific mode of action on the *de novo* purine synthesis in lymphocytes, anaemia due to MMF has been reported (51). Furthermore, occasionally alopecia is encountered with MMF use (41). Inherent to immunosuppressive therapy an increase in infectious complications (viral and opportunistic) and malignancies might be expected, although long-term safety data have to be awaited. Data of two studies with 3-year follow-up gave a slight increase in the incidence of tissue invasive CMV infection and Aspergillus spp in the USA study, which was not the case in the European study(52,53). This difference might be due to the fact that the US trial included induction therapy with anti-T cell monoclonal antibodies. Our own experience is that in individual cases CMV infections following or during MMF treatment can be severe and lead to serious organ involvement (54,55), although in a case-control study no relation between the incidence of CMV infection and MMF exposure was found(56). One study reports an increase in Kaposi's sarcoma, a human herpesvirus 8-associated tumor in MMF treated kidney graft recipients (57). In this study 3/371 MMF treated patients (0.8%), compared to 2/1464 (0.1%) patients not treated

with MMF developed Kaposi's sarcoma. A case report describes the re-occurrence of Kaposi's sarcoma 7 years after transplantation after initiation of MMF (58).

6. Clinical data

Transplantation

Thus far World-wide most experience with the use of MMF exists in solid organ transplantation. MMF is most commonly used as adjunct therapy in combination with calcineurin blockers and prednisone, with or without induction therapy.

Kidney

Three randomised, double-blind, multicenter studies in renal transplant recipients conducted in the USA, Europe and Europe, Canada and Australia were performed, with patient enrolment between 1992-1993 (48) (49) (50). The primary efficacy endpoint in all 3 studies was treatment failure (biopsy-proven acute rejection, graft loss, termination of study and death) within the first 6 months after transplantation. Patients treated with CsA, prednisone and MMF had 50% less acute rejections compared to patients treated with either azathioprine or placebo ($p<0.05$). However, the cumulative 12 month incidence of graft loss and patient death (survival) was not significantly increased for patients treated with MMF.
A pooled analysis of the 3 studies showed a graft survival at 12 months of 90.4% and 89.2% (n.s.) for the MMF patients treated with 2 and 3 g, respectively, compared to 87.6% for the placebo/azathioprine treated patients (n.s.). The incidence of acute rejections was 19.8% and 16.5% for the MMF patients treated with 2 and 3 g, respectively, and 40.8% for the placebo/azathioprine treated patients. For both patient groups treated with MMF, the renal function was consistently better at 3, 6 and 12 months(59).
The 3-year follow-up from the European study demonstrated a 7.6% reduction in the incidence of graft loss (excluding death) for the MMF treated patients(52). A reduced graft loss was not present in the USA study group at 3 years (53). The 5-year follow-up results are expected in the nearby future.
One large randomised study compared maintenance immunosuppressive therapy consisting of tacrolimus and prednisone with tacrolimus, MMF and prednisone (60). Better efficacy was shown for the triple therapy group, with an incidence for acute rejection of 27% vs 44%

(p=0.014). Overall actuarial 1-year patient survival was 94% and overall actuarial 1-year graft survival was 87%, without differences between both groups. These results are comparable to the three studies with CsA as calcineurin blocker combined with MMF and prednisone. Apparently MMF also reduces the incidence of acute rejection in tacrolimus-based immunosuppressive regimens.

In selected patient groups MMF without CsA or a reduced daily dose of CsA has successfully been given to renal transplant recipients from the time of transplantation in an attempt to avoid or lessen unwanted early or late side-effects of the calcineurin blocker (61-64). We performed a randomised study in which stable renal transplant recipients were converted from cyclosporine to MMF or azathioprine at 1-year after transplantation, demonstrating once again that MMF is more efficacious than azathioprine in the prevention of acute rejection (65). Schrama et al also demonstrated beneficial effects of conversion from CsA to MMF in stable renal transplant recipients as soon as 6 months after transplantation, with a lowering of blood pressure, improved glomerular hemodynamics and an improved lipid profile (66).

One steroid sparing study in renal transplant recipients described excess rejection in those patients who were tapered from steroids at 3 months after transplantation compared to patients continuing CsA, MMF and prednisone leading to premature study termination (67). In this study Kaplan-Meier estimated cumulative incidence of rejection or treatment failure within the first year was 9.8% for the maintenance group compared to 30.8% for the steroid withdrawal group. This difference did not translate in a different outcome with regard to patient or graft survival at 12 months after transplantation between both groups. Positive aspects of steroid withdrawal in this study were a lower cholesterol and less anti-hypertensive medication at 6 months after transplantation. A preliminary analysis of the results of a Dutch multi-centre study did not demonstrate a higher incidence of acute rejection in patients withdrawing prednisone 6 months after transplantation compared to patients continuing CsA, MMF and prednisone (68). In this study patients discontinuing CsA experienced a statistically significant increase in acute rejections.

Pancreas-kidney

For combined pancreas-kidney transplantation the combination of MMF with cyclosporine or tacrolimus (FK) compared with azathioprine treated patients with a calcineurin blocker demonstrated similar results as for kidney-transplantation alone (69-73).

Heart

For heart transplant recipients a beneficial effect of MMF on the incidence of acute rejection was found, and here also a reduction in mortality at 1 year, 6.2% vs 11.4% (p=0.031) was found for patients treated with CsA, prednisone and MMF compared to CsA, prednisone and azathioprine, respectively (74). The MMF treated patients also had less requirement of rejection therapy, 65.7% vs 73.7% (p=0.026), experienced less severe rejection (3A), 45% vs 52.9% (p=0.055), and needed less anti-T cell therapy, 15.2% vs 21.1% (p=0.062), compared to the azathioprine treated patients. A parallel conclusion for heart transplant patients with respect to occurrence of acute rejections when patients were converted from MMF to azathioprine late after transplantation was present (75), equal to renal transplant recipients. In this study patients continuing MMF had 4% (1/23) acute rejections compared to 50% (10/20) in patients converted to azathioprine.

Liver

Positive results for MMF combined with calcineurin inhibitors were also found in liver transplantation, both for use in maintenance therapy (76,77) (78), and for rescue therapy (79,80). In maintenance therapy combinations of CsA and MMF vs FK and MMF (historical control) vs FK, MMF and prednisone showed improved graft and patient survival for both FK treated groups, without a statistical significant difference for the patients with triple compared to double therapy including FK (81). Another prospective study demonstrated no significant difference between MMF with either CsA or FK with respect to the incidence of acute rejections and infections, with excellent 6 months graft and patient survival (78). One study compared FK and prednisone with FK, prednisone and MMF treated patients and found a trend for a lower rejection incidence, reduced nephrotoxicity and lower maintenance doses prednisone for the triple therapy group. In this study the actuarial 1-year graft survival was 80.2% vs 79.2% (p=0.77) and patient survival was 85.1% vs 83.1% (p=0.77), for the double vs the triple therapy groups, respectively(77).

Lung

Preliminary results in lung transplantation are also promising for the use of MMF instead of azathioprine. In a non-randomised concurrent cohort study a reduced incidence of biopsy

proven rejection and less decline in FEV_1 after 12 months was found (82). These findings were confirmed by later studies, although all these studies compared prospectively entered patients treated with MMF with historical controls (83-85). A retrospective study compared 3 treatment groups with several immunosuppressive protocols in one centre, group1: r-ATG, CsA, azathioprine, prednisone vs group 2: FK, azathioprine, prednisone vs group 3: FK, MMF, prednisone(86). The incidence of acute rejection per 100 patient days was significantly lower in the FK+azathioprine group ($p=0.045$) and in the FK+MMF group ($p=0.031$) compared to the CsA treated patients, with only a higher rate of freedom from acute rejection for the MMF treated patients. The 1-year actuarial survival rate was 70.6% for the CsA group vs 93% for the FK+azathioprine group ($p=0.044$), with no survival data given for the MMF group because of a comparatively short follow-up. At 3-years after transplantation the actuarial survival rates were 51% and 71%, respectively. The same authors described the same patients with a more extensive analysis, with similar results and conclusions later that year. Additional information was a higher incidence of new onset diabetes mellitus with FK based immunosupression compared to CsA based immunosupression, with similar infection rates(87).

Two studies on salvage therapy using MMF for bronchiolitis obliterans have been published, addressing the efficacy in 1 and 13 patients (88,89). Both papers suggested doses of at least 3 g of MMF as salvage therapy.

Hematology

Graft versus Host disease (GvHD)

For patients treated with an allogenic bone marrow transplantation the danger of developing acute or chronic GvHD is worrisome, and cyclosporine and prednisone are drugs prescribed to prevent GvHD. Positive results in experimental studies led to the use of MMF for the prevention of GvHD (90-92). One study described a salvage effect of MMF and tacrolimus in 46% of patients and the development of stable disease in 11.5% (total patients, n=26)(93). The combination of MMF and cyclosporine has proven successful in the prevention of GvHD in several pilot studies with a steroid sparing effect (94-96). Preliminary results of 4 years experience for the treatment of acute and chronic GvHD with MMF, CsA and prednisone (n=49) compared to CsA and prednisone (n=21), gave a significant overall grade improvement in 72% vs 29% ($p<0.02$) for acute GvHD for the MMF treated patients. A

moderate improvement was present for 7/13 patients with chronic GvHD. MMF resulted in a significant dose reduction of prednisone, and hematological adverse effects were minimal not requiring discontinuation of MMF(97).

Auto-immune thrombocytopenia

Recently the first results of a prospective multi-centre trial including 41 patients with steroid-refractory auto-immune thrombocytopenia and Evans-syndrome were presented(98). In more than half of all patients (22/41) a significant response to MMF treatment was present and in 80% (33/41) of the patients the steroid dose could be safely reduced below 10 mg. During a median follow-up of 17 months (2-26 months), no deaths occurred. The follow-up period was to short to give a prognostic significance to response to MMF therapy, although clinical significance already seems present.

Rheumatology/vasculitis

Rheumatoid arthritis

In addition to commonly used medicaments for patients with rheumatoid arthritis MMF gave a significant clinical improvement in patients refractory to treatment with a variety of disease-modifying anti-rheumatic drugs in one study (99). In this paper it is reported that more than 600 patients received MMF, although actual presentation of data is only given with reference to 2 abstracts including 29 patients. Other authors expect an increased use of MMF as maintenance therapy in rheumatoid arthritis (100,101).

Vasculitis

Although no prospective, randomised studies are presently published yet, preliminary data, several case-reports and experimental (animal) studies favour the use of MMF in auto-immune diseases characterised by vasculitis. Positive effects have been noted in experimental mesangial proliferative glomerulonefritis (rat) (102) and in experimental anti-glomerular basement membrane disease (rat)(103). In nephrotic syndrome patients with divers histopathological diagnoses (n=8), a corticosteroid sparing effect was noted (104). Their primary glomerulonephritis were; membranous (n=3), minimal change (n=2), focal segmental

glomerulosclerosis (n=1), and lupus nephritis (n=2). In 5 children with difficult manageable nephrotic syndrome and evidence of steroid toxicity, 3 patients had a marked clinical improvement, with cessation of corticosteroids in 1 child, after starting MMF treatment(105). These patients had steroid dependent minimal change disease (n=2), partially steroid responsive focal segmental glomerulosclerosis (n=2), and the fifth child did not have a biopsy proven diagnosis.

In a murine lupus nefritis model less binding of immunecomplexes in the glomerular capillary wall was observed with MMF use (106). In two reports SLE patients (n=2 and n=12) refractory to i.v. cyclophosphamide or with relapsing disease were treated with MMF and responded favourably (107,108). At the last meeting of the American Society of Nephrology, November 1999, 2 abstracts reported favourable results, with effective treatment of 5 severe SLE patients resistant to or dependent on cyclophosphamide leading to steroid tapering and 2/5 patients cessation of cyclophosphamide in 1 study (109). In another 6 patients with type IV lupus nephritis MMF treatment resulted in a significantly decreased activity index and change to a less active pathological type of SLE nephritis during a 1-year follow-up period with repeat renal biopsies 6 month after initiation of MMF(110). A report on a prospective, randomised clinical trial gave preliminary results comparing i.v. cyclophosphamide, azathioprine and MMF as maintenance therapy for proliferative lupus nephritis. These authors found similar efficacy for all 3 treatments in proliferative forms of lupus nephritis in 21 patients after successful induction therapy with i.v. cyclophosphamide during a follow-up period of 1-3 years(111).

One case-report claims the disappearance of immunecomplexes, biopsy proven, in immunecomplex glomerulonefritis(112).

Eleven patients with Wegener's disease were succesfully treated with MMF, to avoid long term side effects of cyclophosphamide, after induction therapy with cyclophosphamide (113). The same group reported positive results for IgA nephropathy and systemic vasculitis (114).

A report of 3 therapy resistant patients with Takayasu arteritis with severe vascular involvement dependent on large doses of steroids with resultant toxicity gave impressive results after commencing MMF treatment (115). In 2 patients previous treatment with CsA, cyclophosphamide, methotrexate and azathioprine had failed. All 3 patients had clinical benefit and could be tapered off steroids completely.

Gastro-enterology

M.Crohn

Interest for the use of MMF for the treatment of active inflammatory bowel disease is also present (116). One randomised study compared chronic active M.Crohn patients treated with corticosteroids and azathioprine or MMF, and suggested to switch patients treated with azathioprine who were unresponsive or "allergic" to this treatment to MMF (117). In patients with moderately active disease the same efficacy for MMF or azathioprine was present, whilst patients with highly active disease had an earlier response for the MMF treated patients was present.

Primary biliary cirrhosis

Reports on a positive effect of MMF on liver-enzymes in patients with primary biliary cirrhosis have also been reported (118,119).

Dermatology

Psoriasis was probably the first disease for which MMF treatment was given and efficacy and long-term safety data come from these studies from the 1970's (120,121). At that time the possible immunosuppressive and carcinogenic potentials with the acute gastro-intestinal side-effects led to discontinuation of clinical trials for psoriasis patients. Only the study from Epinette et al followed 85 patients for up to 13 years treated with MMF on a compassionate-use basis. During the first year a mean daily dose of mycophenolic acid of 3705 mg (range:2000-7328 mg) was given which had stabilised at the fifth year of treatment at approximately 3000 mg/day through the twelfth year of therapy. No increase in the incidence of infections or malignancies was observed. More recently interest is increasing again for psoriasis patients with the expanding knowledge and extension of follow-up on safety with MMF. In the literature several case reports with positive results have been published (122) (123,124) (125) (126-128).
Cases with positive results of MMF treatment in patients with bullous skin disease and pyoderma gangrenosum have also been reported (126,129,130).

Neurology

Speculative as it may be, a patient with severe refractory myasthenia gravis has been reported in the literature with a successful response to treatment with MMF(131). The use of azathioprine for multiple sclerosis at doses of 2.5 mg/kg shows marginally significant beneficial results in relapsing-remitting multiple sclerosis but is nevertheless the most widely used global immunosupressive drug for this disease(132-135). Mycophenolate mofetil, with its more specific mode of action and rapidly reversible activity would probably achieve at least the same results. However, proper clinical trials are needed to evaluate such an effect.

7. Conclusion

As is usually the case when a new drug appears on the market with a positive effect in one clinical setting (transplantation), many case-reports with a positive message are published for other indications. Publication bias tends to give less attention to negative results. Thus far mostly positive results in patients with severe, relapsing disease unresponsive to common therapy have been reported with the use of MMF. Proper randomised studies in clinical transplantation have demonstrated a higher efficacy for MMF compared to azathioprine. Because of these findings it is possible that replacement of azathioprine by MMF in a number of diseases will be of benefit for the patient. From this point of view many randomised pilot studies are taking place world-wide and the results of these trials are eagerly awaited.

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Chapter 2

AIMS AND OUTLINE OF THE THESIS

Aims and outline of the thesis

In 1997 mycophenolate mofetil (MMF) was approved for the prevention of acute rejection after kidney transplantation, on the basis of three large randomised trials demonstrating a reduction in the incidence of acute rejection for the combination of MMF, cyclosporine (CsA) and prednisone. In these studies it was shown that the use of MMF in a total daily dose of 3 grams did not result in an additional reduction in the percentage of patients suffering from acute rejection episodes, whilst a daily dose of 3 grams was associated with an increased risk of adverse events. This led to the recommendation to use a standard daily dose of 2 grams MMF.Currently most centres use the combination of CsA, MMF and prednisone in the first months after kidney transplantation, but it is unclear for how long this combination should be continued. Also it is unclear whether the addition of MMF to the immunosuppressive regimen allows the usage of a reduced dose of CsA. Finally, although therapeutic drug monitoring for MMF has not been advised, there are studies demonstrating a relationship between drug concentrations and both efficacy and tolerability. Therefore further research into the value of drug monitoring for MMF is warranted.

The aim of the studies presented in this thesis was to optimise the use of MMF in kidney transplant recipients, in the first 6 months after transplantation, as well as in long term maintenance treatment. In these studies we have investigated whether the use of MMF allows the usage of a reduced dose of CsA during the first 6 months after transplantation and allows the withdrawal of prednisone or CsA from 6 months after transplantation. In stable CsA treated renal transplant recipients we compared efficacy of MMF compared to azathioprine after conversion from CsA to either drug. Furthermore, we have investigated the value of monitoring mycophenolic acid plasma concentrations, with special attention for drug interactions.

Chapter 3

PHARMACOKINETIC STUDIES ON MYCOPHENOLATE MOFETIL

Chapter 3.1

Mycophenolic acid plasma concentrations in kidney allograft recipients treated with or without cyclosporine: a cross-sectional study.

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Abstract

Background. Combining cyclosporine (CsA) and prednisone with mycophenolate mofetil (MMF) results in a significant reduction in the rate of biopsy-proven acute rejection after kidney transplantation. This is achieved with a standard daily MMF dosage of 2 or 3 grams. Whether monitoring of the pharmacologically active metabolite mycophenolic acid (MPA) will lead to improved safety and efficacy is unclear.

Methods. We monitored MPA trough levels in 18 kidney transplant recipients treated with CsA, prednisone and MMF (63 samples) and in 11 patients (31 samples) treated with prednisone and MMF only, in a cross-sectional study. All patients were at least 3 months after transplantation with stable graft function. All patients were treated with 2 g MMF for at least 3 months and 10 mg prednisone.

Results. The MPA trough levels in the CsA-treated patients were significantly lower ($P<0.0001$; Mann-Whitney) than those in patients on MMF and prednisone only (mean MPA levels 1.98 ± 0.12 vs 4.38 ± 0.40 mg/l respectively).

Conclusions. Although all patients were treated with an identical MMF dose, a significant difference was found in the MPA trough levels between CsA- vs non-CsA treated patients. This suggests that CsA influences the MPA trough level. At which level CsA affects the MPA trough levels is unclear.

Introduction

Three large, double-blind, randomized trials have shown that the addition of mycophenolate mofetil (MMF) to an immunosuppressive regimen consisting of cyclosporine (CsA) and prednisone results in a significant reduction in the rate of biopsy-proven acute rejection during the first 6 months after kidney transplantation.[1-3] The size of the reduction in incidence and severity of acute rejection episodes for patients treated with 2 or 3 g MMF was similar; however the 3-g dose was somewhat less well tolerated.[1-4] Therefore, the current daily dose recommendation is 2 g.[5]

Following oral administration MMF is rapidly and essentially completely absorbed and converted to mycophenolic acid (MPA), the active immunosuppressant.[6-7] The sole metabolite of MPA is the glucuronide conjugate MPAG, which is pharmacologically inactive.[6-8] Although clear conclusions have been drawn in regard to clinical efficacy of MMF[1-3], data confirming the usefulness of monitoring MPA concentrations or defining a therapeutic window in terms of

plasma MPA concentrations are not available. So far, the simplicity of fixed dosing (2 g MMF), with the exception of dosing by body size at the extremes in adults and in children[9], is recommended for clinical practice.[6] Results of clinical trials investigating the potential role of therapeutic drug monitoring in MMF treated transplant recipients are not available so far.
Drug interactions with MMF include decreased absorption when coadministered with magnesium and aluminium hydroxide antacids.[5] Cholestyramine decreases bioavailability by interfering with the enterohepatic recirculation.[10] MPA is conjugated to the inactive MPAG.[6-7] CsA is extensively metabolised via the cytochrome P-450 system, an enzyme complex including enzymes having a role in conjugation.[10] No interaction between CsA and MMF has been reported.[10] Tacrolimus and CsA are believed to be metabolised by a common pathway.[11] A recent paper showed higher MPA trough levels and increased AUC_{0-12} values in kidney recipients receiving tacrolimus + MMF compared to patients receiving CsA + MMF.[12] The authors suggested an inhibitory effect of tacrolimus on the conversion of MPA to MPAG to be the mechanism of interaction. However, the data we present in this paper show in fact the CsA-treated patients have relatively low MPA levels.

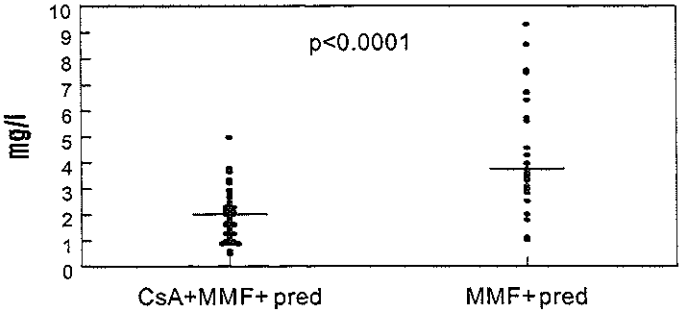
Patients and Methods

In a cross-sectional study we examined the effect of CsA on MPA trough levels. Included were 11 patients treated with MMF and prednisone, 1 year post-transplant , the "non-CsA group". All 11 patients had been on MMF treatment for at least 3 months and had stopped CsA treatment for at least 2 months.
From January 1997 all new kidney transplant recipients are treated with a triple-drug regimen consisting of CsA + MMF + prednisone during the first 6 months after transplantation. These patients form the "CsA group". All MPA samples from the 18 patients in this group were also drawn after treatment with CsA and MMF for at least 3 months. The median time from transplantation was 5 months (3-10) in the CsA group.
Fasted plasma MPA trough levels (EMIT-Mycophenolic Acid Assay, Behring Diagnositics Inc, San Jose, Ca, USA) were routinely measured since February 1997, always 12 h after the previous dose. This immunoassay has been reported to give good agreement with an HPLC assay.[13] For CsA whole blood trough levels also the EMIT immunoassay was used. For both CsA and MPA we participate in the quality assessment scheme from Dr. Holt, St George's Hospital, London.[13]

Results

Figure I shows the clear difference in MPA trough levels between the two groups. In the CsA group MPA trough levels ranged from 0.49 to 4.98 mg/l (mean 1.98 ± 0.12, median 2.02), whereas the range in the non-CsA group was from 1.02 to 9.30 mg/l (mean 4.38 ± 0.40; median 3.75). The difference between the two groups is highly significant (two-sided P-value <0.0001; Mann-Whitney test).

Fig. 1. Mycophenolic Acid trough levels in 18 kidney transplant recipients treated with CsA + MMF + prednisone (n=63 samples) and in 11 kidney transplant recipients treated with MMF + prednisone only (n=31 samples). The difference is statistically significant (P<0.0001 Mann-Whitney test).



The median serum creatinine levels in the CsA treated patients was 118 µmol/l (range 61-246, mean 124) and not different from the non-CsA treated patients (median 110, range 72-213, mean 118, P=0.72). The average prednisone dose in the CsA treated patients was comparable to the non-CsA treated patients (10 mg). None of the patients in either group used any drug known to interact with MMF, nor was a pattern present with any drug being more prominent in either group . The median body weight and height of the CsA vs the non-Csa treated group was: 78 kg (62-92) vs 82 kg (61-100) and 176 cm (159-193) vs 174 cm (153-185), respectively. This was not a statistically significant difference for weight (P=0.5, Mann-Whitney) or height (P=0.7, Mann-Whitney). The intraindividual range for MPA trough levels per patient in the CsA vs the non-Csa treated group is shown in table 1.

Table 1 Fluctuation of MPA trough levels (median+range) for individual patients in the CsA (CsA+MMF+prednisone) and non-CsA treated (MMF+prednisone) groups.

Patient	CsA treated group N=18	non-CsA treated group N=11
1	1.05 (0.98-1.11)	3.43 (2.52-7.55)
2	2.39 (2.11-3.20)	4.30 (3.97-6.41)
3	2.43 (1.98-2.67)	4.11 (3.94-4.28)
4	1.09 (0.88-1.82)	1.02
5	2.02 (1.23-2.37)	1.8
6	1.05	1.79
7	2.1 (2.06-2.70)	5.63 (3.75-7.51)
8	0.57 (0.49-0.92)	5.71 (2.0-7.46)
9	2.31 (1.32-2.64)	3.34 (1.12-4.55)
10	3.74 (3.35-4.98)	8.53 (6.67-9.3)
11	2.77	3.6
12	1.48 (0.88-2.14)	
13	2.39 (1.56-2.96)	
14	2.28 (1.27-2.92)	
15	1.64 (1.12-1.75)	
16	1.22 (0.88-1.92)	
17	2.28 (2.02-2.93)	
18	2.97 (2.24-3.63)	

Discussion

For tacrolimus and CsA most clinicians agree that routine drug monitoring improves the safety and efficacy of these drugs in transplant recipients. Although in kidney transplant recipients a clear reduction in the incidence of acute rejections with MMF has been found[1-4], a further improvement of the outcome using therapeutic drug monitoring still has to be shown. This holds true for MPA monitoring in relation to acute rejection as well as to side-effects.

This paper shows the results of MPA monitoring in two groups of kidney transplant recipients. Although all patients were being treated with a total daily dose of 2 g MMF, a highly significant difference in MPA concentrations was found between the patients treated with CsA+MMF+prednisone and those treated with MMF + prednisone only. How CsA and MMF

interact can not be concluded from this study. Interaction at the level of absorption is unlikely, as bioavailability of MMF is reported to be almost 100% in healthy controls as well as in transplant recipients. However, within the first weeks after transplantation absorption from the gut may be suboptimal in patients with a long history of uraemia. In this study all patients were at least 3 months post-transplantation, making it unlikely that differences in absorption explain the difference in MPA levels. In view of the similar serum creatinines in both groups differences in renal function are also unlikely to be the cause.

Whether there is a certain therapeutic window of optimal MPA levels is unclear so far. A relation has been suggested for the concentration of the MPA level and the amount of immunosuppression, measured by the IMPDH activity which could be seen as an indirect measurement for synthesis of T and B lymphocytes.[14] There are unfortunately no clinical studies published yet which relate MPA levels to rejection rates, to provide tailor-made immunosuppression for the individual patient.

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Chapter 3.2

Effect of cyclosporine on mycophenolic acid trough levels in kidney transplant recipients.

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Abstract

Background. Triple drug treatment consisting of mycophenolate mofetil (MMF), in a standard dose of 2 g daily, combined with cyclosporine (CsA) and prednisone, has become the standard immunosuppressive regimen after kidney transplantation in many centers. The need for therapeutic drug monitoring of mycophenolic acid (MPA) has not yet been established. Several drug interactions with MMF are known. We investigated the influence of CsA withdrawal on MPA trough levels in renal transplant patients.

Methods. Fifty-two patients were treated with 1 g MMF twice daily, and prednisone and CsA targeted between 125 and 175 ng/ml for 6 months after transplantation. At 6 months after transplantation, 19 patients were randomised for continuation of triple therapy (group A), 19 patients discontinued CsA (group B), and 14 patients discontinued prednisone (group C). We compared 12-hr fasted MPA trough levels at 6 and 9 months after transplantation within and between these groups.

Results. MPA trough levels during treatment with CsA, MMF and prednisone were significantly lower than those during treatment with MMF and prednisone only (group B); median levels were 1.87 mg/l (range: 0.56-5.27) vs 3.16 mg/l (range: 0.32-7.78), respectively (P=0.002). MPA trough levels in groups A and C did not change between 6 and 9 months after transplantation; group A median levels were 1.87 (range: 0.31-4.32) vs 1.53 mg/l (range: 0.36-3.70), and group C median levels were 1.62 (range: 0.69-10.34) vs 1.79 mg/l (range: 0.54-6.00), respectively. At 9 months after transplantation, patients in whom CsA was discontinued had higher MPA trough levels as compared with patients who continued the use of triple therapy (P=0.001) or patients in whom steroids were withdrawn (P=0.014).

Conclusion. A significant increase of MPA trough levels was found after discontinuation of CsA (6 months after transplantation), resulting in almost a doubling of MPA trough levels at 9 months after transplantation. This resulted in increased MPA levels in patients without CsA as compared to MPA levels in patients continuing triple therapy or discontinuing prednisone.

Introduction

The addition of mycophenolate mofetil (MMF) to cyclosporine (CsA) and prednisone resulted in a decrease in the incidence of acute rejections during the first 6 months after kidney transplantation [1-4]. Evidence for a positive effect on the occurrence of chronic rejection, either resulting from fewer early acute rejection episodes or as a specific MMF-related effect,

is less convincing. This causes doubts regarding the necessity for long-term continuation of triple therapy in these patients, in view of the possibility of over-immunosupression. MMF is a pro-drug of mycophenolic acid (MPA). The need for therapeutic drug monitoring by measuring trough levels of MPA is unclear, although several arguments favoring therapeutic drug monitoring exist. The randomised concentration-controlled trial has shown a correlation between the area under the curve of MPA levels and the occurrence of acute rejections [5]. In heart transplant recipients the need for therapeutic drug monitoring has also been suggested [6]. Furthermore, there appeared to be a strong correlation between MMF dose and side effects [5,7]. Similar data were found in the three large studies showing a higher incidence of side effects in patients using 3 g compared to 2 g MMF daily [1-4]. Drug interactions with MMF include decreased absorption when coadministered with magnesium and aluminium hydroxide antacids [8]. Cholestyramine decreases bioavailability by interfering with the enterohepatic recirculation [9]. No interaction between MMF and CsA is known [9]. In the present longitudinal study we demonstrate the influence of CsA on MPA levels in kidney transplant patients.

Materials and Methods

From January 1997, fasted MPA 12-hr trough levels were routinely measured in newly transplanted kidney recipients in the University Hospitals of Rotterdam, Nijmegen and Utrecht. For MPA measurements an immunoassay was used (EMIT-Mycophenolic Acid Assay, Behring Diagnostics Inc., San Jose, Ca, USA). Cyclosporine whole blood trough levels were also measured using the EMIT immunoassay or a fluorescence polarisation immunoassay (Abbott Laboratories, North Chicago, IL, USA). For both CsA and MPA, we participate in the quality assessment scheme from Dr Holt, St. George's Hospital, London, United Kingdom [10].

All patients included (n=52), were transplanted after January 1997, and were treated with a standard 2-g daily MMF dose. From 3 months after transplantation, cyclosporine trough levels were targeted between 125 and 175 ng/ml and all patients used 0.1 mg/kg prednisone. Six months after transplantation, 19 patients were randomised for continuation of triple therapy (group A), 19 patients for discontinuation of CsA (group B), and 14 patients for discontinuation of prednisone (group C). In group B, the CsA dose was reduced by 50% for 2 weeks before complete cessation, while increasing the prednisone dose to 0.15 mg/kg and continuing MMF at 2 g daily. In group C the prednisone was tapered off to 0 in 12 weeks,

whilst continuing CsA and MMF. We compared the MPA levels in each group at 6 months (the maximum duration of triple medication before randomisation) and at 9 months (3 months after randomisation) after transplantation.
Representative values of serum albumin, creatinine, bodyweight, cyclosporine daily dose, and 12-hr trough level, were also compared.
For statistical analysis a paired nonparametric comparison within groups was performed (Wilcoxon signed-rank test). When appropriate, a Student's *t* test was performed. For comparisons between different groups a Mann-Whitney test was performed. Results are given as medians with range, unless stated otherwise. A P-value < 0.05 was considered statistically significant.

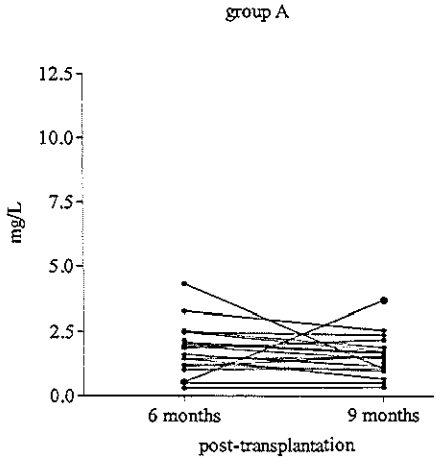
Results

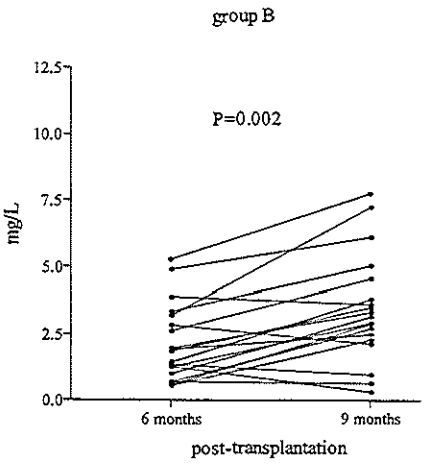
The results of the MPA measurements for the time points 6 and 9 months after transplantation (paired analysis) are shown in figure 1, demonstrating a highly significant difference between the MPA trough levels before and after discontinuation of CsA (P=0.002) in group B.
All patients used 1 g MMF twice daily at both time points. The MPA trough level was nearly twice as high after patients discontinued CsA.
This rise in MPA levels in group B (after cessation of CsA) resulted in significantly higher MPA trough levels compared to those in group A (P=0.001) and C (P=0.014) at 9 months after transplantation. There were no differences in MPA levels between both groups *not* discontinuing CsA. The median values per time points for MPA trough levels between all groups are shown in table 1. The patients in group B had no change in their co-medication in the study-period, eliminating the possibility of other drug interactions.

Table 1 Median 12-hr fasted MPA trough levels for patients using MMF, CsA and prednisone after kidney transplantation. Group A continued triple medication, group B discontinued CsA and group C discontinued prednisone after 6 months.

	6 months MPA (mg/l)	9 months MPA (mg/l)	P
Group A	1.87 (0.31-4.32)	1.53 (0.36-3.70)‡	N.S.
Group B	1.87 (0.56-5.27)	3.16 (0.32-7.78)*‡	0.002
Group C	1.62 (0.69-10.34)	1.79 (0.54-6.00)*	N.S.

Comparison between groups: * P<0.014, ‡ P<0.001





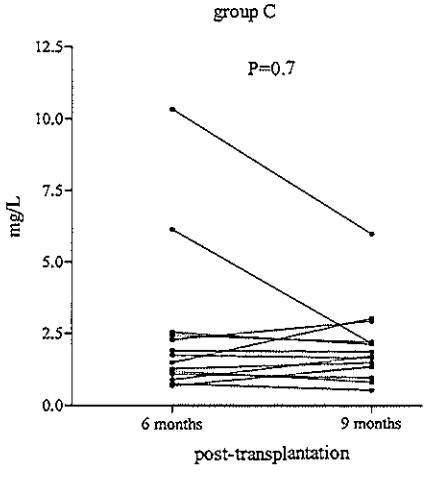


Fig 1 MPA trough levels, at 6 and 9 months after transplantation, in patients continuing triple medication (group A), patients with triple maintenance therapy discontinuing CsA 6 months after transplantation (group B), and patients with triple maintenance therapy discontinuing prednisone 6 months after transplantation (group C)

The results of representative serum albumin and creatinine measurements are shown in table 2. There were no statistically significant changes within each group. Moreover, there were no relevant changes in body weight.

Table 2 Serum albumin, creatinine and body weight for patients using MMF, CsA and prednisone after kidney transplantation. Group A continued triple medication, group B discontinued CsA and group C discontinued prednisone after 6 months. Shown are two time points: 6 months and 9 months after transplantation.

	Group A (n=19)		Group B (n=19)		Group C (n=14)	
	6 months	9 months	6 months	9 months	6 months	9 months
Albumin (g/l)	44 (38-48)	43.5 (37-49)	44 (36-49)	44 (37-52)	43 (37-51)	43.5 (38-48)
Creatinine (μmol/l)	123 (65-411)	119 (60-415)	114 (65-244)	115 (68-179)	136 (82-231)	141 (75-224)
Weight (kg)	83 (60-105)	83 (59-110)	72 (49-87)	73 (50-90)	83 (63-112)	84 (63-116)

At 6 months after transplantation, no differences in CsA trough level or daily CsA dose were present between the three groups (table 3). In both groups continuing CsA a small decrease in daily CsA dose was found, reflected in a somewhat lower CsA trough level at 9 months post-transplantation.

Table 3 Cyclosporine trough levels (mg/l) and daily CsA dose (mg/day) for patients using MMF, CsA and prednisone at 6 and 9 months after kidney transplantation. Data are given as mean with standard deviation. Group A continued triple medication, group B discontinued CsA and group C discontinued prednisone after 6 months.

	6 months	9 months	P
Group A			
trough level (mg/l)	164±39	135±35	0.015
dose (mg/d)	284±76	269±62	0.06
Group B			
trough level (mg/l)	154±39	-	
dose (mg/d)	259±80	-	
Group C			
trough level (mg/l)	158±41	144±34	0.2
dose (mg/d)	282±65	257±55	0.06

Discussion

This paper shows that discontinuation of CsA increases the MPA levels in renal transplant patients. Previously, we demonstrated a comparable difference in MPA trough levels between patients with and without CsA, although that cross-sectional study was performed in two different groups at different intervals after transplantation [11]. To strengthen this finding, we measured MPA levels within patient populations over time, comparing these levels just before and 3 months after discontinuing CsA from 6 months after transplantation. MPA levels in patients continuing triple medication and in patients discontinuing prednisone were also measured for comparison.

Only MPA levels from 6 months after transplantation were used to avoid influence of malabsorption from the gut and a possible influence of rising MPA levels, which has been proposed to occur during the first months after transplantation [5].

No changes in renal function or serum albumin could be found, factors that could also possibly interfere with MPA levels. The increase in MPA levels in patients who discontinued the use of CsA (group B) strongly suggests a lowering effect (interaction) of CsA on MPA trough levels.This is further supported by the difference in MPA levels between patients with CsA (group A and C) and without CsA (group B) at 9 months after transplantation. An explanation for the reduction of MPA levels in patients with concomitant CsA use is not readily apparent.

The bio-availability of MMF is reported to be almost 100%, excluding differences in resorption between the groups as a plausible explanation. There are, however, only limited data on the bio-availability of MMF with the use of CsA, as most studies on this issue have been performed with MMF only in healthy volunteers.

Another possibility might be an influence of CsA on the enterohepatic recirculation, although this is also speculative, as there are no data available supporting this theory. Cyclosporine, originally developed to be used as an antibiotic, might decrease the amount of glucuronidase producing gut flora leading to less deglucuronidation of the MPA glucuronide, which is excreted in bile. After cessation of CsA, there could be a restoration of the gut flora, resulting in increased deglucuronidation and subsequently an increased amount of MPA available for reabsorption.

The EMIT assay used for MPA measurements is thought to cross-react with an acyl glucuronide metabolite (MPAG) of MPA [12,13]. A rise in MPAG as a result of CsA withdrawal is unlikely, as renal function did not alter when CsA was discontinued and withdrawing CsA nephrotoxicity would decrease rather then increase our observed effect on MPA levels.

In the literature an interaction between MMF and another calcineurin blocker (tacrolimus), has been reported [14]. In this study patients receiving tacrolimus and MMF 2 g/day displayed significantly higher MPA levels than those receiving CsA and MMF. The authors ascribe this difference to an effect of tacrolimus on MPA levels, although the findings are more in agreement with an effect of CsA, when this and our study are taken together. The MPA levels in their patients not treated with CsA (MMF and tacrolimus), are comparable to the MPA levels in group B in our study, whereas the MPA levels in their patients receiving CsA are comparable to those in our patients continuing CsA (groups A and C).

An interaction at the level of the cytochrome p 450, which is involved in the metabolism of CsA is less likely, as no interaction between MMF and drugs which are known to induce the cytochrome p 450 system has been reported.

In summary, this is the first description of an effect of CsA withdrawal on sequential MPA levels in stable kidney transplant recipients. The clinical relevance of this finding has to be determined.

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Chapter 3.3

Mycophenolic acid trough levels after kidney transplantation in a cyclosporine-free protocol.

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Abstract

Twenty-seven stable kidney transplant recipients treated with cyclosporine (CsA) and prednisone, were converted to mycophenolate mofetil (MMF) and prednisone 1 year after transplantation. After conversion the patients were treated with a standard daily dose of 1g MMF b.i.d. and 10 mg prednisone for 4 months. Thereafter, two MMF dose reductions were performed with a 4-month interval. Mycophenolic acid (MPA) trough levels were measured at regular intervals. A relation was found between MPA trough levels and MMF dose. The median MPA trough level for patients treated with 1 g MMF b.i.d. was 4.3 μg/ml (0.95-15.5) and 3.0 μg/ml (0.73-7.8) for patients treated with 750 mg b.i.d. (p=0.0002). The MPA trough levels further decreased from 3.0 μg/ml to 2.3 μg/ml (0.6-6.63) in patients treated with 500 mg MMF b.i.d. (p=0.01). Dose reduction of MMF from 1 g to 750 mg b.i.d. could be performed without acute rejections. A further dose reduction to 500 mg b.i.d. elicited 3 rejections. Patients experiencing an acute rejection had a median MPA trough level of 2.3 μg/ml (1.26-3.38) compared to 3.8 μg/ml (1.48-6.52) in patients without an acute rejection (P=0.25). We conclude that there is a significant relation between MPA trough levels and MMF dose. MPA trough levels were not predictive of rejection in the present study.

Introduction

At present the combination of cyclosporine (CsA), mycophenolate mofetil (MMF) and prednisone is the standard immunosuppressive drug regimen after kidney transplantation in most centres. The recommended dose for MMF is 1 g b.i.d., while therapeutic drug monitoring is not advised by the manufacturer. However, others have stressed the importance of therapeutic drug monitoring [1]. Moreover, these recommendations are based on the use of MMF in combination therapy with CsA and prednisone from the time of transplantation. Whether such a strategy also holds true in patients not treated with CsA can only be speculated. We demonstrated a significant difference in mycophenolic acid (MPA) trough levels between patients treated with or without CsA in combination with MMF and prednisone, resulting in almost twice as high MPA trough levels in patients discontinuing CsA [2,3]. Therefore, it is reasonable to assume that higher MPA trough levels might reflect an increased area under the curve (AUC), i.e. immunosuppression. Considering this, lowering

the MMF dose might be possible without an increased risk for the occurrence of an acute rejection. This paper describes the results of dose reduction and MPA trough levels in renal transplant patients treated with MMF and prednisone.

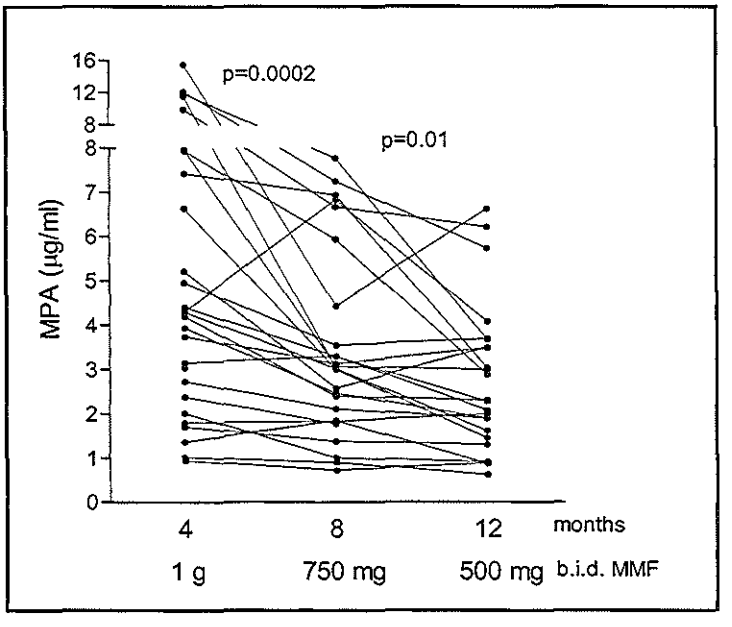
Patients and methods

We performed a prospective study including a cohort of 27 stable kidney transplant recipients, who were transplanted between September 1995 and January 1997 and treated with cyclosporine (CsA) and prednisone for one year. Hereafter, these patients were converted to mycophenolate mofetil (MMF) with a standard daily dose of 1 g b.i.d., without altering the prednisone dose (10 mg). Four and 8 months after this conversion a dose reduction of MMF was performed, resulting in a daily dose of 750 mg and 500 mg b.i.d., respectively. The end of follow-up for analysis was one-year after conversion. Fasted mycophenolic acid (MPA) 12-hour trough levels were measured at outpatient visits. For MPA measurements an immunoassay was used (EMIT-Mycophenolic Acid Assay, which was kindly provided by Dade Behring Inc., San Jose, Ca, USA.). For this assay, we participate in the quality assessment scheme from Dr Holt, St. George's Hospital, London [4]. Four 12-hour area under the curve (AUC_{0-12}) were performed, two patients treated with 1 g and two patients with 500 mg MMF b.i.d., respectively. Clinical and laboratory examinations were routinely performed during outpatient visits. MPA trough levels at the maximum time of duration, just before dose reduction, were used for comparison (4, 8 and 12 months after conversion). In the event of an acute rejection, the closest pre-rejection MPA trough level was used and compared to two MPA trough levels from patients converted at approximately the same time, with similar MMF dose as control. Results are given as medians with range or means with standard deviation, unless stated otherwise. Paired and unpaired comparisons of numerical data were performed using Wilcoxon's signed ranks and Mann-Whitney tests, respectively. A P value <0.05 was considered significant.

Results

A significant relation was found between MPA trough levels and MMF dose, when comparing all individual MPA levels at 4, 8 (p=0.0002) and 12 months (p=0.01) after conversion (figure 1).

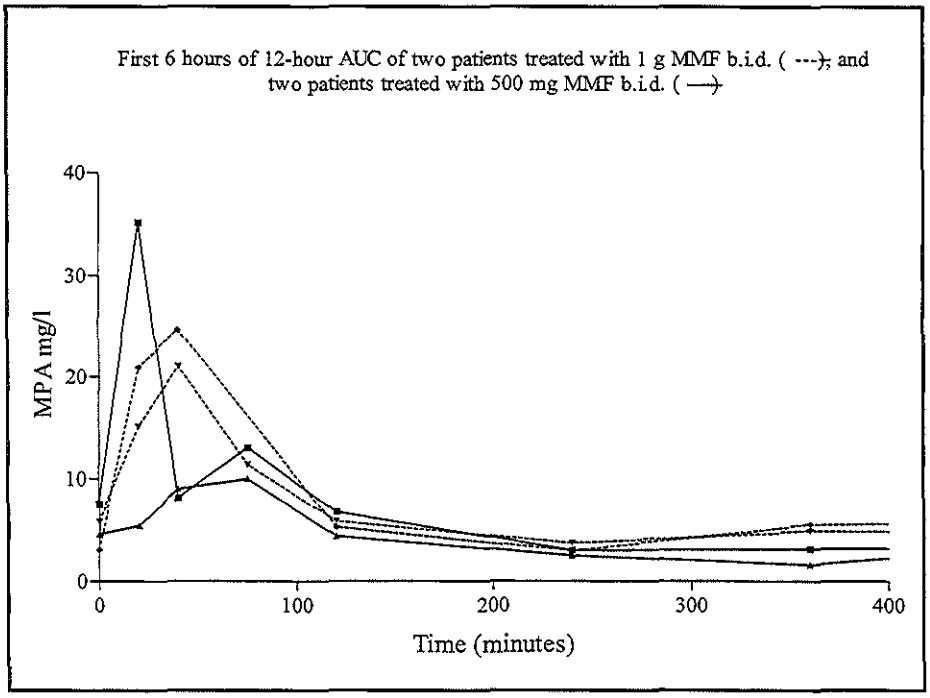
Fig. 1 Relation between fasted 12-h mycophenolic acid (MPA) trough levels and mycophenolate mofetil (MMF) dose



The first 6 hours from the 12-hour AUC's for four patients are shown in figure 2. The mean AUC_{0-12} was 98.2±38.0 vs 58.2±8.4 µg.hr/ml for 1 g vs 500 mg MMF b.i.d., respectively. No relation between the AUC and MPA trough levels was present in the limited number of patients studied.

Acute rejection occurred in three patients after the second dose reduction.

Fig.2 Abreviated AUC of two patients treated with either 1 g or 500 mg MMF twice daily



There was no significant difference between the median MPA trough levels in the three patients experiencing an acute rejection; 2.3 μg/ml (1.26-3.38) compared to the controls 3.8 μg/ml (1.48-6.52; p=0.25). Patients with high MPA trough levels (>3.5 μg/ml) had no signs of rejection.

There was no deleterious effect on renal function after the first MMF dose reduction, when comparing serum creatinines at 4 months (112.5 μmol/l) vs 8 months (112.1 μmol/l) after conversion (p=0.52). However, the serum creatinine at 12 months after conversion had increased significantly compared to 8 months from 112.1 μmol/l to 123.4μmol/l (p=0.0164). This increase was attributable to three patients: two patients had a recurrence of their original kidney disease and one patient had chronic rejection. If the analysis was censored for these patients, the serum creatinine was 112.1 μmol/l at 8 months and at 12 months 113.4 μmol/l (p=0.08). If the analysis was censored for the 3 patients with acute rejection, the serum creatinine was 111.1 μmol/l at 8 months and at 12 months 119.4 μmol/l (p=0.05).

Discussion

The dilemma facing the clinician in the management of the renal transplant recipient is finding the lowest possible immunosuppressive regimen without endangering the graft. We describe the results of a sub-analysis, focusing on the possibility of MMF dose reduction and the relation with MPA trough levels. MMF dose reduction from 1 g to 750 mg b.i.d. was uneventful, whereas a further dose reduction of MMF to 500 mg b.i.d. was accompanied by three acute rejections in 27 patients. A decrease in renal function was found for the last 4 months of follow-up, which was attributable to chronic rejection (n=1) and recurrence of the original kidney disease (n=2). When the goal of maintenance immunosuppression is to achieve zero acute rejections, a policy of reducing the dose of MMF to 750 mg b.i.d. seems to be safe. With regard to therapeutic drug monitoring a significant relation between MMF dose and MPA trough level was found for individual patients. However, no clear relation could be demonstrated in the patients with rejection when comparing median MPA trough levels to controls as defined in the methods section. It must, however, be stressed that only a small number of rejections occurred. Moreover, AUC could be a better index for impeding rejection than trough level. Indeed, although only a limited number of AUC's were performed, a difference between patients treated with either 1 g or 500 mg MMF b.i.d. seems to exist especially in the first 6 hours of the total 12-hour AUC.

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Chapter 4

CLINICAL STUDIES ON MYCOPHENOLATE MOFETIL

Chapter 4.1

Elective withdrawal of mycophenolate mofetil in renal transplant recipients treated with mycophenolate mofetil, cyclosporine and prednisone.

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Abstract

In a retrospective study we investigated the risk of acute rejection after withdrawal of MMF in 39 adult patients treated with cyclosporine (CsA), prednisone and mycophenolate mofetil (MMF) for at least 6 months following renal transplantation. After reaching a stable renal graft function, MMF was withdrawn and CsA and prednisone were continued. Preceding the withdrawal of MMF, four patients experienced an acute rejection. During a median follow up of 38 months after discontinuing MMF no acute rejection occurred. The mean serum creatinine level did not change during the first six months after withdrawal of MMF. We conclude that elective withdrawal of MMF in stable renal transplant recipients at 6 months after transplantation bears no important risk for the occurrence of acute rejection.

Introduction

The addition of mycophenolate mofetil (MMF) to the immunosuppressive treatment with cyclosporine (CsA) and prednisone results in an important reduction in the incidence of acute rejection during the first half-year after renal transplantation (RTx) (1,12,14,17). However, improvement in graft survival has not convincingly been demonstrated with continued treatment of MMF for three years after RTx (2,9,15). The risks of over-immunosuppression with regard to increased susceptibility to infections and malignancies are well known. Therefore, a reduction in the amount of immunosuppressive drug treatment seems desirable once stable engraftment of the kidney has been reached. Withdrawal of CsA or steroids from triple drug therapy consisting of CsA, azathioprine (AZA) and steroids has been associated with the occurrence of acute rejection episodes (6,7), which might influence long term outcome. Currently, no data concerning the risk of rejection after stopping of MMF are present. The aim of our study was to investigate the risk of acute rejection after withdrawal of MMF in RTx patients who were initially treated with MMF in combination with CsA and prednisone.

Methods

We performed a retrospective study including 39 adult patients who received a renal graft from a cadaveric or living donor at the University Hospitals of Nijmegen and Rotterdam (the Netherlands) between June 1994 and November 1998. All patients were treated with the combination of CsA, prednisone and MMF for at least 6 months following transplantation, and all patients had a stable renal function. Twenty-one patients participated in a dose finding study of MMF (17). The MMF dose varied between 1000 and 4400 mg (median 2000 mg) in these patients. In eight of these patients, treated in one participating centre, MMF was stopped at 6 months after transplantation. In thirteen of these 21 patients, treated in the other centre, the decision to stop MMF treatment was arbitrarily made at a median time of 12 months after RTx (range 6-26 months). The remaining 18 of the 39 patients received a standard dose of 2000 mg of MMF. These patients also stopped MMF at 6 months after transplantation, which was our standard protocol for patients who did not participate in a clinical trial requiring continuation of this drug. Besides the MMF dose and duration, the immunosuppressive therapy and other basic characteristics were similar in patients treated with MMF for six months or in patients in whom MMF was discontinued at a later time, so we decided to analyse these patients as one group. In 23 patients it was decided to stop treatment with MMF at once and in the remaining 16 patients the MMF dose was tapered to zero in several weeks. CsA (target trough level 100-300 ng/ml) and prednisone (0.10-0.15 mg/kg) were continued. In 17 patients it was decided to increase the prednisone dose slightly to 0.15 mg/kg/day, which was the standard dose in one of the participating centres. The CsA dose and target level were not changed. The analysis was restricted to patients with a duration of follow up of more than six months after stopping of MMF.

Acute rejection was defined as a rise in serum creatinine levels in combination with the histologic evidence of acute rejection, which necessitated the use of anti-rejection treatment.

Serum creatinine levels, haemoglobin levels, leukocyte counts and thrombocyte counts were assessed at baseline (2 months before and at the day of stopping or reducing MMF), and 2 and 6 months after withdrawal of MMF.

Statistical analysis

Results are presented as medians with ranges or as means with standard deviations. Comparison of the numerical data between baseline and after follow up was performed using the Wilcoxon's signed rank test. For correlation analysis, a Spearman rank test was used. A p-value smaller than 0.05 was considered statistically significant.

Results

The baseline characteristics of the 39 patients are shown in table 1. Four patients had experienced an acute rejection during the first 6 months after RTx, while using triple therapy. Three of these patients were treated with 1 gram methylprednisolone intravenously on three consecutive days and one patient with anti-T-cell therapy. MMF was withdrawn more than 5 months after anti-rejection treatment in these patients. In two of these patients the prednisone dose was subsequently increased from 0.12 mg/kg/day to 0.15 mg/kg/day.

Table 1 Characteristics of patients in whom MMF was stopped after renal transplantation (n=39).

Sex (Male / Female)	15 / 24
Median age (years)	48 (18-67)
Mismatches on HLA-A and HLA-B ≤1 / ≥2	8 / 31
" HLA-DR 0 / 1	17 / 22
Re-transplantation	5
Median time of withdrawal of MMF after RTx (months)	6 (6-26)
Patients with acute rejection before withdrawal of MMF	4
Median duration of follow up after stopping MMF (months)	38 (6-59)

The median duration of follow up after withdrawal of MMF was 38 months. During this period none of the patients experienced an acute rejection. There was no change in serum creatinine level or in proteinuria during the first half year after withdrawal of MMF (Table 2). Leukocyte counts increased significantly after stopping MMF. The same was true for haemoglobin levels, although a stable situation had not been reached at the time of MMF withdrawal.

During the first two months after stopping MMF the median increase in body weight was 1.0 kg (range –3.9 to +8.0 kg; $p<0.01$). There was no correlation between the MMF dose and the increase in body weight after stopping MMF or between the increase in body weight and the increase in prednisone dose after stopping MMF. The weight adjusted MMF dose correlated weakly with the increase in leukocyte counts after withdrawal of MMF ($r=0.3$, $p<0.05$).

Table 2 Clinical and laboratory parameters at baseline (-2 months and T0) and 2 and 6 months after stopping MMF, respectively.

	-2 months	T0	+2 months	+6 months
CsA trough level (ng/ml)	167(96-360)	171(95-360)	162(59-290)	170(86-330)
Prednisone dose (mg/kg)	0.12(0.09-0.2)	0.12(0.09-0.2)	0.14(0.09-0.2)	0.13(0.08-0.2)
Creatinine (µmol/l)	119±32	118±32	116±30	120±34
Proteinuria >0.5 g/l (n)	4	3	1	1
Haemoglobin (mmol/l)	7.8±1.1[#]	8.1±1.1	8.5±1.0[#]	8.5±1.1[#]
Leukocytes ($x10^9$/l)	7.7±2.1	7.7±2.3	8.8±1.5[#]	9.2±1.8[#]
Thrombocytes ($x10^9$/l)	240±63	232±62	229±61	244±61

[#]$P<0.01$ versus T0

Discussion

From this retrospective study we conclude that elective withdrawal of MMF bears no important risk for the occurrence of acute rejection in stable renal transplant patients. None of the patients in our study group experienced an acute rejection after stopping MMF. It is important to recognise that these patients had a stable renal graft function at 6 months or more after transplantation and as such form a selected population. Only 10% of the patients had an acute rejection before withdrawal of MMF, while the incidence of acute rejection in our hospital was approximately 25% in all patients treated with MMF, CsA and prednisone. In comparable cohorts of patients treated with the combination of MMF, CsA and prednisone the incidence of acute rejection varied between 17% and 20% (1,12,14,17). Thus the low rejection incidence in our study population might be explained by selection due to exclusion of patients with unstable renal

graft function. On the other hand, a previous rejection episode in a patient with a stable renal function was no reason to continue MMF in our hospitals.

The best procedure to withdraw MMF is not clear from our data. MMF dose was tapered to zero with a concomitant increase in prednisone dose to 0.15mg/kg body weight in approximately 40% of the study group. The relevance of these measures is probably minor because rejections neither occurred in the rest of our study group. Our data do not allow firm conclusions on the risk of late chronic rejection after withdrawal of MMF. However, during at least half a year after withdrawal of MMF, renal function remained stable and there was no increase in proteinuria.

The body weight increased slightly after withdrawal of MMF, while it had been stable during the two months before. Use of MMF is related with gastrointestinal complaints, and the weight gain might reflect increased appetite after stopping of MMF. Haemoglobin level and leukocyte counts increased slightly after stopping MMF. This seems to reflect some bone marrow suppression induced by MMF. Notably, the haemoglobin level already increased during the baseline period, so other factors besides withdrawal of MMF may play a role. Adverse effects of MMF may be related to higher trough levels of mycophenolic acid, the active metabolite of MMF (11,16), although other data indicated a dose relationship between MMF dose and the occurrence of side effects (17). The limited variation in MMF dose (69% of our patients used a standard dose of 2 grams per day) reduced the possibility to detect a relationship between MMF dose and severity of side effects. Nevertheless, a weak correlation was found between the MMF dose and the increase in leukocyte count after stopping MMF.

MMF is a very valuable drug for reducing the incidence of acute rejection in the first period after RTx. After this period the risk of acute rejection is considerably lower, possibly due to a certain degree of immunologic adaptation to the renal graft (8). The additional benefit of MMF in preventing acute and chronic rejections once the host immune response to the graft has adequately been suppressed, has not been demonstrated. On the other hand, the risks of over-immunosuppression are well known. Over-immunosuppression is related with increased risk of (opportunistic) infections and development of malignancies (10). MMF in particular seems to increase the incidence of symptomatic CMV infections (13), and has been related with human herpes 8 virus infections and consequently Kaposi sarcoma (5). The risk of development of other malignancies during long term treatment with MMF is not known at this moment. A reduction in the amount of maintenance immunosuppression is desirable as long as it does not negatively

influence long term outcome. At the time of this study no data concerning the risks of withdrawal of CsA or prednisone from triple therapy consisting of CsA, prednisone and MMF were available. However, withdrawal of CsA or prednisone from triple drug therapy consisting of CsA, prednisone and AZA has been associated with an increased incidence of acute rejection episodes (6,7), which might negatively influence long term renal graft survival. Withdrawal of AZA from this triple drug regimen at more than six months after RTx is associated with a low incidence of acute rejection (0-5%) (3,4). We therefore chose to withdraw MMF and to continue CsA and prednisone in our stable patients, which appeared to be a safe procedure with no acute rejection episodes at all.

In conclusion, these data suggest that treatment with MMF can safely be stopped in renal transplant patients with a stable graft function at six months or more after RTx.

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Chapter 4.2

Randomized study on the conversion of treatment with cyclosporine to azathioprine or mycophenolate mofetil followed by dose reduction

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Abstract

Background The introduction of cyclosporine (CsA) in kidney transplantation has improved early graft survival. However, its long-term use is associated with impairment of renal function and increased cardiovascular risk factors. To avoid CsA-related long-term adverse effects, patients were converted to either azathioprine (AZA) or mycophenolate mofetil (MMF) 1 year after transplantation.

Methods Between September 1995 and January 1997, 64 stable renal transplant recipients on CsA and prednisone treatment were included in a prospective, randomized study. Patients were randomized for conversion of CsA to 2 mg/kg AZA (n=30) or 1g MMF twice daily (n=34). All patients remained on low-dose steroids. To decrease the total immunosuppressive load, a dose reduction in MMF and AZA was performed at 4 and again at 8 months after conversion. Mycophenolic acid trough levels were measured at regular intervals

Results After conversion a decrease in serum creatinine was found for both groups: for MMF, 132 to 109 μmol/l (P=0.016) and for AZA, 123 to 112 μmol/l (P<0.0001). After conversion, more acute rejections occurred in the AZA group (11/30) compared to the MMF group (4/34), (P=0.04). Dose reduction of MMF to 500 mg twice daily and of AZA to 1.0 mg/kg elicited three rejections in both groups. The incidence of side effects and infections were similar.

Conclusion Discontinuation of CsA spared renal function. In patients converted to MMF significantly less rejections occurred compared to patients converted to AZA. Furthermore, dose reduction of both AZA and MMF is possible in the majority (72%) of the patients.

Introduction

The introduction of cyclosporine (CsA) as maintenance immunosuppressant in kidney transplantation has markedly improved early graft survival. The long-term outcome of kidney transplant patients is influenced by the occurrence of chronic rejection and cardiovascular disease [1-3]. Unwanted side effects of CsA such as hyperlipidemia and hypertension exacerbate the risk for developing cardiovascular disease [4,5]. Patients receiving renal replacement therapy have a 15- to 20-fold increased risk of myocardial ischemia and infarction when compared with age- and sex-matched healthy controls [6,7]. Moreover, maintaining a high immunosupressive load is associated with a high incidence of malignancies on the long term [8]. Cardiovascular mortality accounts for 16-36% of all deaths

in renal transplantation and 9-12% of all deaths are due to malignancy [United States Renal Data System 1999].

In an attempt to improve the long-term survival of graft and patient, and to avoid CsA-related adverse effects, conversion from CsA to azathioprine (AZA) in stable renal transplant recipients can be performed at different time points after transplantation [9-13]. These conversions carry a risk of acute rejection, which by itself might influence the long-term graft survival [14,15]. However, this was refuted in three studies, which compared outcome more than 5 years after transplantation [16-18]. Meanwhile, clinical trials in kidney transplantation showed better efficacy for maintenance therapy with a combination of CsA, mycophenolate mofetil (MMF) and prednisone compared to CsA, AZA and prednisone [19-22]. We therefore decided to perform a study in stable renal transplant recipients 1 year after transplantation, treated with CsA and prednisone, comparing the effect of conversion to either MMF or AZA with prednisone. Inasmuch as a high immunosuppressive load contributes to infectious complications and malignancies in long-term renal transplant patients, a dose reduction in the AZA and MMF group was performed 4 and 8 months after conversion.

Materials and methods

Between September 1995 and January 1997, 104 kidney transplantation were performed in Rotterdam. Sixty-four renal transplant patients with stable graft function 1 year after transplantation, without acute rejections within the last 6 months and treated with CsA and prednisone, agreed to participate. We performed a prospective, randomized study examining the effect of conversion either to MMF and prednisone (n=34) or to AZA and prednisone (n=30). This study was approved by the hospital ethics committee, and all patients included gave informed consent. The end of follow-up was February 1st, 1999.

To decrease the total immunosuppressive load, two dose reductions were performed 4 and 8 months after conversion, resulting in a 50% dose of MMF or AZA at 8 months after conversion. The starting dose of MMF was 1 gram twice daily and 2 mg/kg/day for AZA. At the time of conversion, the CsA dose was gradually tapered and stopped after 4 weeks, without altering the daily prednisone dosage of 10 mg. The frequency of postconversion follow-up was decided by the controlling physician who saw the patient at the out-patient clinic. If patients experienced side effects, a further dose reduction did not occur until the protocol-driven reduction was set to take place, or on explicit patient's request, or when deemed absolutely necessary by the treating physician . If patients experienced acute

rejection, a further dose reduction was only performed in those patients who responded to the anti-rejection therapy with a return of the serum creatinine to prerejection value. No preconversion biopsies were taken to asses the presence of chronic rejection or cyclosporine nephrotoxicity. For the patients randomized for conversion to MMF, fasted mycophenolic acid (MPA) 12-hr trough levels were measured. For MPA measurements an immunoassay was used (EMIT-mycophenolic Acid Assay, kindly provided by Behring Diagnostics Inc., San Jose, Ca, USA). For this assay, we participate in the quality assessment scheme from Dr Holt (St. George's Hospital, London, United Kingdom) [23]. MPA trough levels were not used for dose adjustments of MMF. When relating MPA levels with side effects or acute rejections in patients treated with MMF and prednisone, the MPA level at the time of the event or the closest pre-event MPA level was taken. Two MPA trough levels from a patient without side effects or acute rejection in the same group converted at a comparable point in time, with the same MMF dose were used as a control.

Statistical analysis

First, we performed an analysis on basis of intention-to-treat; and, second, we analyzed the data of follow-up using the assigned maintenance immunosuppressive medication. For both analyses, the occurrence of rejections, infections, malignancies, mortality, and sequential serum creatinine between both conversion groups were compared. The occurrence of side effects and 24-hr proteinuria was analyzed for patients using the assigned study medication. Endpoints for the intention-to-treat analysis were end of follow-up, graft loss (dialysis), or death. Endpoints for analysis with the assigned maintenance immunosuppression were end of follow-up or a switch in medication. Numerical data were compared using paired-t, Mann-Whitney U or Fisher's exact tests where appropriate. Unless stated otherwise results are reported as the median with range or the mean with standard deviation. A two-tailed P-value of <0.05 was considered to be significant.

Results

The baseline characteristics of both groups of patients at the time of conversion are shown in table 1. No statistically significant differences were observed between the two groups. The primary renal diseases were also comparable in both groups.

Patients were followed until February 1st, 1999, with a total follow-up after conversion for the intention-to-treat analysis of 1.61 ± 0.6 vs 1.72 ± 0.54 years for the patients in the MMF vs the AZA group. In patient years, this follow-up was 54.6 and 51.72 for the MMF vs AZA

group, respectively. The median follow-up on the assigned treatment after conversion of 1.50 years (0.25-2.25) vs 1.53 (0.46-2.27) for the patients in the MMF vs the AZA group, with a total of 41.4 and 33.3 patient years for the MMF vs AZA group, respectively.

Table 1. Baseline characteristics of the patients at the time of randomization to AZA or MMF and prednisone 1 year after kidney transplantation.

	MMF+prednisone (n=34)	AZA+prednisone (n=30)
Sex (female/male)	15/19	12/18
Age (yr)	46 (21-73)	44 (22-67)
Living/postmortal donor	10/24	8/22
Average No. of mismatches:		
HLA-A	0.7±0.8	0.6±0.7
HLA-B	0.8±0.6	0.7±0.7
HLA-DR	0.4±0.5	0.5±0.5
Cold ischemia time (hr)	20'23 (1'30-31'18)	18'12 (1'30-34'27)
CMV disease 1st year	9	11
Acute rejections 1st year	10	7
CsA trough level (μg/l)	145 (80-260)	162.5 (110-350)
Serum creatinine (μmol/l)	131.5 (80-281)	123 (42-238)

Endpoints

In the MMF/prednisone group, 24 patients were able to adhere to the study-protocol including full dose-reduction, and 10 patients changed the assigned maintenance immunosuppressive medication. In these 10 patients changing the assigned maintenance immunosuppressive medication, CsA was restarted in 4 patients because of side effects and in 2 patients after rejection, while 2 patients increased their dose to 1 g twice daily after rejection. In one patient, a fulminant pancreatitis necessitated discontinuation of his assigned medication. There was graft-loss for one patient in the MMF group as a result of untreatable acute rejection, which was elicited by a to rapid decrease of MMF from 1 g to 500 mg twice daily necessary because of severe leucopenia ($1.3x10^9$ leucocytes).

In the AZA/prednisone group, 20 patients were able to adhere to the study-protocol including full dose reduction, while 10 patients changed the assigned maintenance immunosuppressive

medication. In these 10 patients changing the assigned maintenance immunosuppressive medication, cyclosporine was restarted in 2 patients because of side effects and in 7 patients after rejection. One patient in the AZA group discontinued his assigned medication because of an anti-glomerular basement glomerulonephritis.

One patient in the AZA group was lost to follow-up from 9 months after randomisation, after uneventful full dose reduction.

Three patients in the AZA group died; no deaths occurred in the MMF group (p=0.36). All three patients had a functioning graft. The causes of death were: lung carcinoma, pulmonary embolus and cerebrovascular accident. The patient with the cerebrovascular accident used CsA, AZA, and prednisone; the other two patients used the assigned maintenance immunosuppressive medication.

Dialysis was restarted in four patients in the MMF group; the patient who had graft loss because of rejection, one patient had recurrence of his original kidney disease (uneventful full dose-reduction), and two patients who had restarted CsA because of side effects early after conversion. There were no patients in the AZA group who restarted dialysis (p=0.12).

Immunosuppressive medication

For 22 patients, the reduction of MMF resulted in a daily dose of 750 mg twice daily after 4 months and 500 mg twice daily at 8 months until the end of follow-up. The 17 patients in the AZA group started with an average daily dose of 150±27 mg, which was reduced to 111±26 mg (P<0.001), and to 80±18mg (P<0.001) at 4 and >8 months after conversion, respectively.

Renal function A marked improvement in serum creatinine was present 2 months after conversion to either MMF or AZA (table 2).

Table 2. Median serum creatinine (range) before and after conversion from CsA and prednisone to MMF and prednisone or AZA and prednisone.

	MMF+prednisone creatinine (μmol/l)	AZA+prednisone creatinine (μmol/l)
conversion	131.5 (80-281)*	123 (42-238)‡
2 months	106 (73-263)*	103 (40-208) ‡
6 months	108.5 (75-186)	111 (47-186)
12 months	109 (71-206)	112 (55-187)

Comparison within groups: * P=0.016, ‡ P<0.0001

For the patients using the assigned maintenance immunosuppressive medication, this effect continued during the follow-up period. When analyzing renal function according to the intention-to-treat principle, thus including patients who were not able to adhere to the assigned study medication, no significant differences were present between the MMF group (211 μmol/l; range, 72-964) and the AZA group (124 μmol/l; range, 48-269) at the end of follow-up, respectively.

Rejection

After conversion from CsA and prednisone, several patients developed an acute rejection in both groups (table 3) in different time periods; during the first 4 months after conversion, 4-8 months after conversion (1st dose reduction), and >8 months after conversion (2nd dose reduction). The severity of these rejections, measured by the amount and type of anti-rejection therapy, were different: 14 rejections responded to one course of methylprednisolone (5 MMF, 9 AZA), and 5 rejections responded to two courses of methylprednisolone (1 MMF, 4 AZA). In both groups, one patient required ATG treatment within 4 months after conversion. This resulted in graft loss for the patient in the MMF group. One patient who had been converted to MMF switched back to CsA and prednisone developed an acute rejection thereafter. For the intention-to-treat analysis this resulted in a total of 8 vs 14 rejections (p=0.22) for the MMF and AZA group, respectively.

Table 3. Number of acute rejections after conversion and dose reduction from CsA and prednisone to MMF or AZA and prednisone per patient at risk.

Time after conversion	MMF+prednisone rejections/patients	AZA+prednisone rejections/patients	P
0-4 months (conversion)	4/34	11/30	0.04
4-8 months (1st reduction)	0/29	3/25	n.s.
>8 months (2nd reduction)	3/26	0/20	n.s.

There was no relation between the occurrence of acute rejection in the first year after transplantation (before conversion) and acute rejection after conversion compared to those patients not experiencing early acute rejections (P=0.48). Nor was there a relation between the severity of rejection before conversion (first year after transplantation) and the occurrence of acute rejection after conversion (P=1.0), or the number of acute rejections before conversion and the occurrence of acute rejection after conversion (P=0.55). Whether the serum creatinine

at the time of conversion was high or low (above or below the group mean value) could not be related to the occurrence of acute rejection after conversion (P=1.0). The patients receiving a living related kidney did not differ in the risk of acute rejection after conversion compared to patients receiving a postmortal kidney (P=0.74). There was also no relation between acute rejection after conversion and cytomegalovirus disease in the first year after transplantation (P=1.0).

Chronic rejection (biopsy proven) was found in five patients during follow-up (three MMF, two AZA). Recurrence of the original kidney disease was present in four patients (three MMF,one AZA).

In 22 patients a 24-hr proteinuria>500 mg (15 MMF/7 AZA) was present at the end of the study period. The presence of proteinuria after conversion was related to chronic rejection in four patients (three MMF/one AZA) and to recurrence of the original kidney disease in four patients (three MMF/one AZA). In the MMF group, 2 patients developed de novo proteinuria and 13 patients were already proteinuric at the time of conversion. In the AZA group, four patients developed de novo proteinuria.

Side effects

There was no statistically significant difference in the total number of side effects between the patient groups (table 4). Hematological side effects were anemia and/or leucopenia in both groups. Gastrointestinal side effects occurred in the period with the maximal daily dose of MMF or AZA. Alopecia became apparent between 4 and 8 months after conversion of CsA, in patients converted to either MMF or AZA.

Table 4. Side effects after conversion and dose reduction from CsA and prednisone to MMF or AZA and prednisone per patient at risk.

Side-effect	Hematological		Gastro-intestinal		Alopecia	
Time after conversion	(event/patient)		(event/patient)		(event/patient)	
	MMF	AZA	MMF	AZA	MMF	AZA
0-4 months (conversion)	5/34	9/30	6/34	2/30	0/34	0/30
4-8 months (1st reduction)	3/29	5/25	2/29	0/25	4/29	5/25
>8 months (2nd reduction)	1/26	0/20	0/26	0/20	0/26	1/20
Total	9‡	14‡	8**	2**	4	6

Comparison between groups; ‡P=0.12, **P=0.09

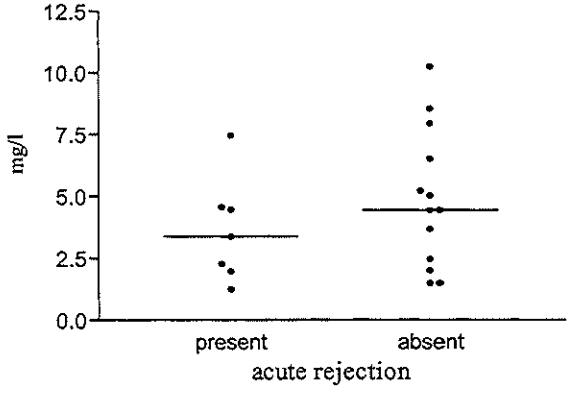
Infection/malignancies

For patients with the assigned maintenance immunosuppressive medication, the number of infections per patient-year was equally distributed between both groups, 0.44 (20/41.44) vs 0.27 (9/33.25) in the MMF and AZA group (P=0.27), respectively. The number of infections per patient-year, for the intention-to-treat analysis, was also equally distributed between both groups, 0.47 vs 0.38 in the MMF and AZA group (P=0.60), respectively. In the MMF group, one patient, who had received r-ATG 1 week after transplantation, developed a lymphoma 2 years after transplantation. This patient had strikingly high MPA trough levels (mean 8.3±3.3, median 7.5 [range, 5.12-14.81]) during follow-up despite dose reductions of MMF. Two malignancies developed in the AZA group: one cervical carcinoma in situ and one lung carcinoma. The latter patient died as a result of this malignancy.

Therapeutic drug monitoring

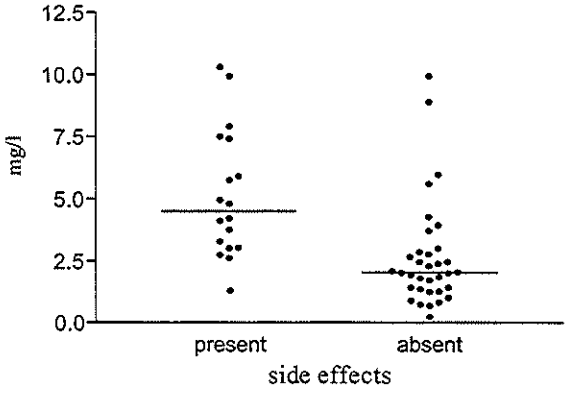
The median MPA level for patients with acute rejection was 3.4 versus 4.4 mg/l (P=0.34) for patients without acute rejections, respectively (figure 1). For one patient no prerejection MPA trough level was present. In 86% of the patients experiencing an acute rejection, MPA trough levels were below 5 mg/l.

Fig. 1 Relation of mycophenolic acid levels and acute rejection (P=0.34, Mann-Whitney)



The median MPA level for patients with side effects was 4.5 versus 2.0 mg/l (P=0.0001) for patients not experiencing side effects, respectively (figure 2). Adequate MPA trough levels could not be found for two patients in the control group.

Fig. 2. Relation of mycophenolic acid levels and side effects (P=0.0001, Mann-Whitney)



Discussion

The addition of MMF to CsA and prednisone in kidney transplantation has markedly reduced the incidence of acute rejections in the early posttransplantation period, with a better efficacy for MMF compared to AZA when combined with CsA and prednisone [19-22]. Limited data exist on the use of MMF and prednisone as maintenance immunosuppression in kidney transplantation [24,25]. Zanker et al converted 13 patients treated with CsA, selected on clinical grounds for having nephrotoxicity, to treatment with MMF monotherapy. This conversion was not complicated by acute rejection in any of their patients and led to an immediate and long-lasting improvement of renal function [25]. Our study is the first randomized study that describes the results of elective conversion from CsA to MMF in renal transplant recipients, with conventional conversion from CsA to AZA as controls. Furthermore, in order to reduce the overall immunosuppressive load, we aimed for a long-term maintenance therapy that was lower than conventional full-dose treatments.
A marked improvement of the serum creatinine after conversion to either MMF or AZA was observed when CsA was discontinued. This effect was comparable to results in previous studies with AZA [16-18] and the study with MMF [25]. Patients randomised to AZA had a higher risk of an acute rejection after conversion than patients randomised to MMF. The relatively high risk for acute rejection after conversion may cause concern regarding the application of this strategy to large numbers of patients, but it should be noted that less than 10% of these rejections required anti T-cell antibody therapy and that only one rejection caused graft loss. Although this undesirable consequence of conversion in the short term may well be outweighed by beneficial changes in the long term, like an improvement of renal

function, improvement in cardiovascular risk profile and reduced immunosuppressive load in the majority of patients. Furthermore, a low threshold for performing biopsies in these patients (increase of serum creatinine of 10%) might have overrated the incidence of acute rejection. Also, it might be conceivable that lowering CsA to very low doses or tapering CsA over a longer period before conversion might decrease the incidence of acute rejection. A therapeutic window for drug monitoring for patients treated with MMF and prednisone, cannot be established yet, because of the small number of acute rejections occurring with different MMF doses, making a clear recommendation concerning therapeutic drug monitoring in the setting of dual therapy with MMF and prednisone not possible on the basis of this study. However, a clear relationship between MPA trough levels and a number of side effects (leucopenia, anaemia, alopecia) was present.

There was an equal distribution in the number of patients with biopsy-proven chronic rejection between both groups (three MMF/ two AZA). A possible explanation might be that a positive effect of MMF on chronic rejection only occurs when MMF is given from the start of transplantation. The 3-year follow-up of the Tricontinental Trial showed a trend (not statistically significant), toward a better graft and patient survival for patients using MMF instead of AZA, although this study was not designed to establish a significant difference in these outcome variables [26].

The incidence of de novo malignancies in both treatment groups was equally distributed. The patient in the MMF group who developed a lymphoma had strikingly high MPA levels, which might have contributed to the occurrence of this malignancy. This is, however, speculative, because ATG treatment was also given to this patient during the early posttransplant period. Cardiovascular mortality was only present in the AZA group.

Should all patients be converted to MMF and prednisone given the results of our study? Patients who suffer from suspected CsA-related side effects such as CsA nephrotoxicity, hypertension, or hyperlipidemia are good candidates for conversion to MMF and prednisone. Furthermore, patients with low donor-specific T-cell reactivity can also be safely converted, as described by van Besouw et al [28]. No other risk factors or patient characteristics could be identified from the first year after transplantation, which might predict the occurence of acute rejection after conversion, i.e., in whom conversion would be contraindicated. All rejections occurred in a short time after conversion or dose reduction. This fact does not make us feel anxious about acute rejections with a longer follow-up. No analysis with regard to cost effectiveness of the two regimes was performed in this study. MMF is more expensive than AZA, but this price difference might be overruled by the fact that patients with MMF have

less acute rejections and therefore less anti-rejection treatment and less complications requiring hospital admission. The cost effectiveness of MMF(1 g twice daily) in the first year after primary cadaveric transplantation compared to a standard regimen that included AZA (1-2 mg/kg per day), has been demonstrated before [27]. However, long term cost effectiveness will need to be determined when data are available of the effect of MMF on long term graft function, repeat transplantation and patient survival.

We conclude that conversion from CsA and prednisone to MMF and prednisone at 1 year after kidney transplantation is at least as safe and as effective as conversion to AZA and prednisone. Furthermore, a dose reduction of both MMF and AZA is possible in a substantial number of patients.

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Chapter 4.3

A controlled trial comparing two doses of cyclosporine in conjunction with mycophenolate mofetil and corticosteroids

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Abstract

It is unknown whether the addition of mycophenolate mofetil (MMF) to cyclosporine (CsA) and prednisone after renal transplantation (RTx) allows for a reduced dose of CsA, in order to minimize the incidence of CsA-related side effects and to reduce costs. Therefore, 313 renal allograft recipients were randomized for treatment with MMF 1000 mg bid, prednisone, and either conventional or low dose CsA during the first three months after RTx. The target trough levels were 300 and 150 ng/ml respectively during the first three months, and 150 ng/ml in both groups thereafter. 313 patients were included: 161 patients received conventional dose and 152 low dose of CsA. During the first 6 months after transplantation, graft failure or patient death occurred in 19/161 patients (11.8%) in the conventional CsA group and in 11/152 patients (7.2%) in the low CsA group (ns). Biopsy proven acute rejection occurred in 36/161 patients (22%) in the conventional CsA group and in 29/152 patients (19%) in the low CsA group (ns). The incidence of delayed graft function was similar in both groups (31/161 (19%) vs 28 /152 (18%); ns). Serum creatinine did not differ between the conventional and the low CsA group: 151 ± 56 μmol/l vs 142 ± 49 μmol/l at 3 months and 141 ± 60 μmol/l vs 136 ± 49 μmol/l at 6 months. There were no differences between the groups regarding blood pressure, lipid metabolism, and infectious complications. In the low dose group, an estimated $500 per patient was saved on the costs of CsA. In conclusion, the addition of MMF to CsA and prednisone after renal transplantation allows the use of a lower than conventional dose of CsA, without increasing the risk of rejection.

Introduction

The addition of mycophenolate mofetil (MMF) to a standard immunosuppressive regimen consisting of cyclosporine (CsA) and prednisone has led to a major reduction in the rejection rate during the first six months after renal transplantation [1-4]. In the three so-called "pivotal" trials, designed to investigate the efficacy of MMF when added to standard therapy, patients were treated with a full dose of CsA. Although there was no clear increase in the incidence of side-effects related to over-immunosuppression, the results gave rise to concerns regarding the long-term safety of this triple drug regimen [1;2]. One possible way to reduce the risk of over-immunosuppression would be to lower the standard dose of CsA. If this would not

hamper the efficacy of the triple drug regimen, it might have the additional benefit of a reduction in CsA-related side effects such as nephrotoxicity, hypertension, and delayed graft function, which frequently complicate the course after renal transplantation. With a reduced CsA dose, the differentiation between rejection and CsA nephrotoxicity also might be easier. Finally, a reduction of the CsA dose might partly compensate for the increase in costs of medication, caused by the addition of MMF.
In this multicenter, open-label trial, recipients of a renal allograft were randomized to treatment with a conventional dose or with a low dose of CsA, in combination with MMF and prednisone.

Patients and methods

Adult recipients of a first or second renal transplant from a living related or cadaveric donor were eligible for this study. Excluded were recipients of a graft from an HLA-identical living related donor or a non-heart-beating donor, patients with liver function disturbances, peptic ulcer, diarrhoea, leucocytopenia or thrombocytopenia, patients with a hemolytic uremic syndrome as original renal disease, women not using adequate contraception, and patients taking immunosuppressive medication other than corticosteroids at the time of transplantation.
The study design was approved by the institutional review boards of the three participating hospitals, and written informed consent was obtained from all participants prior to transplantation.
Immunosuppression
During the first two post-operative days, CsA was given intravenously in a dose of 3 mg/kg/day in the conventional dose group and 2 mg/kg/day in the low dose group. From the third postoperative day, CsA was given orally (starting dose 10 and 6 mg/kg/day respectively) and was dosed to reach a target trough level during the first three months of 300 ng/ml (250-350) in the conventional dose group and of 150 ng/ml (125-175) in the low dose group. From 3 to 6 months after transplantation, the target trough level was 150 ng/ml in both groups. Interruption of CsA treatment was allowed for a maximum of 28 days. The micro emulsion formulation of CsA (Neoral$^{(R)}$) was used in all patients. CsA whole blood levels were measured with a monoclonal antibody against the CsA parent molecule, using the FPIA assay

on an Abbott TDx analyzer (Abbott Laboratories, North Chicago, IL, USA) or an EMIT assay on a COBAS-MIRA analyzer (Dade-Behring, San Jose, CA, USA).

MMF was administered in a fixed dose of 1000 mg bid. Dose reduction or interruption of MMF treatment was allowed in cases of leucocytopenia, primary CMV infection or severe gastro-intestinal side effects. If the dose of MMF was reduced to less than 1000 mg/day during more than 14 days, this was considered a violation of the treatment protocol.

Prednisone was given 100 mg iv during the first three days, followed by an oral dose of 0.4 mg/kg/day from day 4-14 and then tapering gradually to 0.1 mg/kg/day at three months; this last dose was continued thereafter. Induction therapy with anti T-cell preparations was not used.

Rejections were treated primarily with methylprednisolone 1000 mg iv for three consecutive days. In case of steroid-resistant rejection, anti T-cell therapy was given (either rabbit polyclonal antithymocyte globulin (ATG) or the mouse anti-CD3 monoclonal antibody WT32 [5]). If patients in the low dose group needed anti T-cell treatment during the first three months after transplantation, they were subsequently treated with the conventional dose of CsA.

Additional medication

All patients received prophylaxis for peptic ulcers (famotidine 20 mg or ranitidine 150 mg once daily) and pneumocystis carinii pneumonia (cotrimoxazole 480 mg once daily). CMV prophylaxis with ganciclovir or CMV hyperimmune globulin was prescribed during anti T-cell therapy in patients at risk for CMV disease (donor and/or recipient seropositive).

Assessments

At baseline, the medical history, physical examination, routine laboratory tests, lipid profile and histocompatibility data were obtained. Every month vital signs, body weight, and the results of routine laboratory measurements were recorded. Data on rejection episodes, CsA nephrotoxicity and dialysis requirements, concomitant medication, adverse events, and infections were recorded throughout the entire study period. A biopsy was performed in cases of deteriorating graft function without an obvious pre- or postrenal cause. No protocol biopsies were performed.

Biopsies were examined by the local nephropathologist and were classified according to the Banff 1993 biopsy scoring system (borderline, grade 1 (=mild), grade 2 (=moderate) and grade 3 (=severe) rejection) [6]. Delayed graft function was defined as the need for one or more dialysis sessions, more than 24 hours after transplantation. For calculation of the creatinine

clearance the 24 hours urinary creatinine excretion and serum creatinine were measured. Infections were classified using the CDC definitions for nosocomial infections [7]. CMV disease was defined as "mild" in cases of fever for more than three days with leuco- or thrombocytopenia or liver function disturbances; in cases of proven organ localization, CMV disease was defined as "severe".

A questionnaire assessing the severity of known side effects of immunosuppressive drugs on a semiquantitative scale was completed by the patient at 1, 3, and 6 months (see appendix).

Randomization procedure

Shortly before transplantation, patients were randomly assigned to one of the treatment groups in a 1:1 ratio, with stratification for cadaveric / living related transplant and for center. Randomization was carried out by opening a sealed envelope with the lowest available study number.

Endpoints

The primary endpoints were the incidence of biopsy proven acute rejection (Banff grade 1 or higher) and of CsA nephrotoxicity during the first three months after transplantation. CsA nephrotoxicity was defined as an otherwise unexplained rise in serum creatinine of more than 15% above the previous level, which was reversible after lowering the CsA dose. Secondary endpoints included: time to first acute rejection, number of acute rejections within the first three months, number of biopsies, the incidence and duration of delayed graft function, and graft function at 1 and 3 months. All end points were also assessed at 6 months after transplantation.

Statistical analysis

Results are given as means ± standard deviation unless stated otherwise. The statistical analysis was performed on an intention-to-treat basis.

Comparison of continuous variables between the groups was performed using the Wilcoxon rank sum test. Categorical variables were analyzed with the Chi squared test. Comparison of time to first rejection was performed using the Kaplan-Meier procedure with Log Rank testing. A p-value < 0.05 was considered significant. Calculations were performed using the SAS system, version 6.12 (SAS Institute, Cary, NC, USA) and Graphpad Instat $^{(R)}$, version 3.00 for Windows (Graphpad Software Inc., San Diego, CA, USA).

Results

Between January 1, 1997 and December 31, 1998, 313 patients were enrolled. The demographic data of these patients are summarized in Table 1; no significant differences existed between the groups.

CsA levels

During the first three months, mean CsA levels exceeded the target levels in both groups, despite frequent dose reductions (Table 2). Nevertheless, in accordance with the design of the study, CsA levels were significantly different between both groups during the first three months after transplantation. However, some overlap in CsA levels between the groups existed, with approximately 25% of levels in the conventional dose group falling below the 75th percentile of the low dose group during these first three months. Despite a similar CsA dose in both groups from three months after transplantation, CsA levels remained slightly higher in the conventional dose group. At 6 months after transplantation, both CsA dose and CsA level were not significantly different between both groups (Table 2).

Table 2. CsA level and dose during the first six months after transplantation (median and 25th -75th percentile).

	CsA level (ng/ml)			CsA dose (mg/kg/day)		
	Conventional	Low	P	Conventional	Low	P
Week 1	310 (250-400)	182 (145-240)	0.0001	9.7 (7.9-10.1)	6.1 (5.6-6.8)	0.0001
Week 2	340 (260-420)	188 (150-240)	0.0001	8.3 (6.3-10.0)	5.7 (4.6-6.7)	0.0001
Week 3	340 (260-450)	200 (160-250)	0.0001	7.4 (5.7-9.5)	5.2 (4.1-6.7)	0.0001
Month 1	325 (255-405)	194 (160-240)	0.0001	6.8 (5.4-7.8)	5.1 (4.1-6.5)	0.0001
Month 2	275 (230-325)	170 (140-210)	0.0001	5.5 (4.4-6.9)	4.3 (3.3-5.3)	0.0001
Month 3	248 (200-290)	154 (130-180)	0.0001	4.9 (4.0-6.5)	4.1 (3.0-4.9)	0.0001
Month 4	180 (150-220)	150 (130-170)	0.0001	4.0 (3.3-5.2)	3.9 (3.1-4.7)	0.1
Month 5	160 (140-190)	152 (130-170)	0.03	3.7 (3.1-4.9)	3.7 (3.0-4.5)	0.35
Month 6	156 (130-190)	150 (130-175)	0.18	3.7 (3.1-4.6)	3.8 (2.9-4.5)	0.33

Table 1. Patient and donor characteristics.

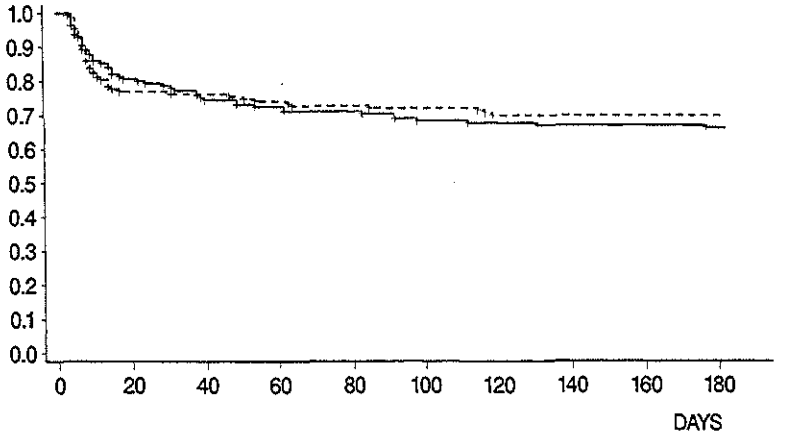
	Conventional CsA	Low CsA
N	161	152
Sex (M / F)	98 / 63	96 / 56
Age (years)	48 ± 14	49 ± 14
Weight (kg)	73 ± 14	72 ± 12
Primary disease		
Chronic glomerulonephritis	27 %	33 %
Chronic pyelonephritis	12 %	8 %
Cystic disease	16 %	16 %
Diabetes mellitus	7 %	7 %
Renovascular disease	2 %	4 %
Other	22 %	18 %
Unknown	14 %	14 %
Haemodialysis / CAPD / No dialysis	85 / 62 / 14	80 / 58 / 14
Historical maximum PRA (Median, Range)	5 (0 - 100)	5 (0 - 100)
First / Second graft	138 / 23	132 / 20
Cadaveric / Living donor	117 / 44	116 / 36
Donor sex (M / F)	95 / 66	93 / 59
Cadaveric Donor age (years)	46 ± 17	42 ± 18
Living Donor age (years)	46 ± 12	49 ± 13
HLA mismatches	2.5 ± 1.3	2.4 ± 1.3
Preservation fluid (UW / HTK / Eurocollins / other)	107 / 35 / 18 / 1	94 / 37 / 21 / 0
Cold Ischemia Time (hours)*	24 ± 7	25 ± 7
Anastomosis time (minutes)	31 ± 10	33 ± 13

* Cadaveric kidneys only

Rejections and biopsies

The incidence of biopsy proven rejection within the first six months was 36 / 161 (22%) in the conventional CsA dose group and 29 / 152 (19%) in the low CsA dose group (ns). The histological severity of these biopsy proven rejections was not different between the groups (Table 3). Moreover, the median time to the first acute rejection episode was similar in both groups: 15 and 9 days respectively (ns) (figure 1).

Fig. 1 Freedom from acute rejection between the conventional (—), and low CsA dose group (- - -).



The incidence of presumed rejections was similar in both groups (conventional dose 15 / 161 (9%) and low dose 10 / 152 (7%)), as was the incidence of borderline biopsies in the biopsy (7 / 161 (4%) and 8 / 152 (5%) respectively) (Table 3).

Treatment for a biopsy proven or a presumed rejection was given in 58 / 161 (36%) patients in the conventional dose and in 45 / 152 (30%) patients in the low dose group (ns) (Table 3). In all but four cases, first line anti rejection treatment consisted of a course of methylprednisolone. Treatment with one course of methylprednisolone was sufficient in 38 / 58 (66%) and 30 / 45 (67%) of the cases in the two groups. In 4 and 1 patients, additional corticosteroids were administered. In 14 / 58 (24%) and 12 / 45 (27%) patients,

methylprednisolone treatment was followed by anti T-cell therapy because of steroid-resistant rejection. Two patients in each group were primarily treated with anti T-cell therapy.
In 11 patients in the conventional dose group and in 12 patients in the low dose group one or more subsequent rejection episodes occurred; in 8 and 9 cases respectively, these second rejections were biopsy proven. Anti T-cell treatment for a recurrent rejection episode was needed in 5 patients in each group.

Graft failure, patient death and protocol failure

Within 6 months after transplantation, graft failure occurred in 14 / 161 (8.7%) patients in the conventional CsA group and in 8 / 152 (5.3%) patients in the low CsA group (ns). The reasons for graft loss were not different between groups and included ongoing rejection (conventional dose group 4, low dose group 2 cases), vascular thrombosis (5 and 2 cases), primary nonfunction (2 and 4 cases), and other causes (3 and 0 cases).
Patient death with functioning graft occurred in 5 / 161 (3.1%) patients in the conventional dose group and in 3 / 152 (2.0%) patients in the low dose group (ns). Causes of patient death were not different between groups and included cardiovascular events (2 vs 1), infections (1 vs 0) and other reasons (2 vs 2).
In 27 / 161 (17%) patients in the conventional CsA group and in 20 / 152 (13%) patients in the low CsA group, cessation or interruption of one or more of the immunosuppressive drugs was judged necessary for clinical reasons. CsA was discontinued in 15 and 11 patients, MMF in 6 and 8 patients, CsA plus MMF in 4 and 1 patients, and prednisone in 2 and 0 patients respectively. In 6 / 27 and 3 / 20 patients with treatment failure, graft failure or death with functioning graft occurred subsequently (ns).
Altogether, treatment with the combination of CsA, MMF and prednisone was continued during the 6 month study period in 121 / 161 (75%) patients in the conventional CsA and 124 / 152 (82%) patients in the low CsA group (ns).

Table 3. Rejection episodes during the first 6 months after transplantation. There are no statistically significant differences.

	Conventional CsA (n = 161)	Low CsA (n = 152)
Biopsy proven rejection	36 / 161 (22%)	29 / 152 (19%)
0-3 months	31 / 36	27 / 29
3-6 months	5 / 36	2 / 29
Presumed rejection*	22 / 161 (14%)	18 / 152 (12%)
0-3 months	21 / 161	17 / 152
3-6 months	1 / 161	1 / 152
Histological severity of biopsy proven rejection (0-6 months)		
Grade 1 rejection	20 / 36 (56%)	16 / 29 (55%)
Grade 2 rejection	16 / 36 (44%)	10 / 29 (35%)
Grade 3 rejection	0 / 36 (0%)	3 / 29 (10%)
Time to first rejection episode** (days)	15 (4-177)	9 (3-119)
Treatment of first rejection episode**	58 / 161 (36%)	47 / 152 (30%)
Corticosteroids only	42 / 58 (73%)	31 / 47 (66%)
Corticosteroids followed by anti-lymphocyte agent	14 / 58 (24%)	12 / 47(26%)
Initial antilymphocyte agent	2 / 58 (3%)	2 / 47 (4%)
No treatment	0 / 58 (0%)	2 / 47(4%) ***

* Presumed rejection: patients treated with methylprednisolone without performing a graft biopsy, and patients treated with antirejection therapy while the biopsy showed only borderline rejection or no signs of rejection
** Rejection episodes includes biopsy proven as well as presumed rejections
*** In two cases in the low CsA group, there were histological abnormalities indicating acute rejection (one rejection grade 1 and one borderline rejection) but graft function improved spontaneously without treatment

Graft function and blood pressure

The incidence of delayed graft function was equal in the conventional and the low dose group: 31 / 161 (19%) vs 28 / 152 (18%) patients (ns). The median duration of dialysis treatment in these patients was also similar in both groups: median 7 days (ranges 1-35 and 1-55 days respectively; ns). At all time points during follow-up, serum creatinine as well as calculated creatinine clearances, and proteinuria were comparable in both groups (Table 4).

According to the criteria given in the methods section, episodes of CsA nephrotoxicity occurred in 13 / 161 (8%) patients in the conventional CsA and 4 / 152 (3%) patients in the low CsA group (p=0.06).

Episodes of graft dysfunction that resulted in performing a biopsy tended to occur more frequently in the conventional CsA group (number of biopsies per patient: 0.65 vs 0.49; p=0.09). To examine whether episodes of CsA induced graft dysfunction might have contributed to this finding, we compared the biopsy results between both groups. The number of biopsies showing no histological abnormalities, which is compatible with the presence of the acute, reversible form of CsA induced renal dysfunction, was higher in the conventional CsA group (13% vs 1% of all biopsies; p=0.02)

Throughout the study period, there were no differences between the groups regarding blood pressure or the number of antihypertensive drugs (Table 4).

Table 4. Graft function and blood pressure at 3 and 6 months after transplantation.

	3 months			6 months		
	Conv*	Low	P	Conv	Low	P
Creatinine (μmol/l)	151 ± 56	142 ± 49	ns	141 ± 60	136 ± 49	ns
Creatinine clearance (ml/min)	59 ± 32	66 ± 36	ns	65 ± 28	69 ± 31	ns
Proteinuria (g/day)	0.6 ± 1.2	0.7 ± 2.0	ns	0.5 ± 0.6	0.5 ± 0.6	ns
Proteinuria >1 g/day (N=)	16	19	ns	19	17	ns
Mean arterial pressure (mm Hg)	106 ± 11	103 ± 13	ns	104 ± 12	103 ± 12	ns
Antihypertensive drugs (N=)	1.2 ± 0.8	1.2 ± 0.8	ns	1.2 ± 0.9	1.3 ± 0.9	ns

* Conv = Conventional

Safety and costs

Within the first 6 months after transplantation, there were no cases of post-transplant lymphoproliferative disease or other malignancies.

The number of infections per patient was equal in both groups (1.7 ± 0.7 per patient). The most frequent infections were urinary tract infections (34 and 38 episodes), oral candidiasis (14 and 12 episodes) and CMV infection. The overall incidence of CMV disease was 31 / 161 patients (19%) in the conventional dose group and 35 / 152 patients (23%) in the low dose group (ns). When analyzed per serological donor-recipient combination, there were also no differences between the two groups in the incidence or the severity of CMV disease.

During the first 6 months after transplantation, there were no significant differences between the conventional and the low dose group in total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and Lp(a).

The number of patients experiencing at least one recorded adverse event was similar in both groups: 74% and 78% (ns). Diabetes mellitus developed in 6 patients in both groups. Liver function disturbances were rare, occurring in less than 2% in both groups.

The questionnaire revealed no significant differences in the incidence or in the severity of drug-related side effects (Table 5).

Table 5. Questionnaire - Percentage of patients noticing a specific side effect at a certain time point (Most frequent side effects only; ie >50% of patients reporting the side effect).

	Month 1		Month 3		Month 6	
	Conv*	Low	Conv	Low	Conv	Low
Fatigue	75	67	69	63	54	52
Hirsutism	39	35	80	84	76	81
Swollen face	56	55	59	63	47	45
Tremor	64	64	62	56	48	40

* Conv = Conventional

An estimation of the savings on the costs of CsA, based on an actual price of $ 4 / 100 mgr CsA, revealed that cost savings in the low dose group amounted to approximately $ 500 during the first 6 months after transplantation, which means a reduction by 20%.

Discussion

The results of this prospective trial indicate that in renal transplant patients treated with the combination of CsA, MMF and prednisone, prescribing a lower than usual dose of CsA does not increase the incidence or the severity of acute rejections. Previous studies have demonstrated that in the setting of double therapy with steroids or triple therapy with azathioprine and steroids, lower CsA levels are associated with an increased risk of acute rejection [8;9]. Our findings therefore suggest that the more recently introduced immunosuppressive drug MMF can exert a so-called 'CsA-sparing' effect. Recently, a similar CsA-sparing effect was observed when rapamycin was added to the combination of CsA and steroids [10]. Although CsA levels were measured frequently and doses were repeatedly adjusted to reach the desired range, mean CsA trough levels in both groups somewhat exceeded the target, especially during the first three months. Apparently, to reach trough levels of CsA of 300 and 150 ng/ml respectively, the starting dose of CsA should be lower than was prescribed in our protocol (10 and 6 mg/kg/day). Nevertheless, in agreement with the aim of the study protocol, there was a significant and potentially meaningful difference in CsA levels and doses between the two groups during the first three months. Furthermore, nearly 80% of all included and analyzed patients were treated according to the study protocol during the full six months. Taken together, it seems unlikely that insufficient adherence to the study design has obscured a conceivable detrimental effect of a lower target CsA level on the rejection incidence.

The incidence of biopsy proven acute rejection in our study population (22 and 19%) seems to be slightly higher than in the three so-called 'pivotal' clinical trials with MMF, where an incidence of acute rejection of 17-20% was found [1-3]. Several factors might be responsible for this difference: the use of induction therapy [1], a considerably higher corticosteroid dose [1;3], a much higher CsA dose, or a combination of these factors [1;2] in these three studies. However, a concise comparison with regard to these factors cannot be made, as the exact dosing schedules of prednisone and CsA were not described in two of these three studies. Nevertheless, despite

this slightly higher rejection incidence in our study, patient and graft survival in our study were comparable to the survival data in the three 'pivotal' studies.

When designing the study protocol, we expected to find a difference in the incidence of CsA-related side effects between the treatment groups. Several studies indicate that the avoidance of CsA during the first days after transplantation by treating the patients with induction therapy with anti-lymphocyte antibodies, results in an earlier recovery of delayed graft function and an overall better graft function [11]. The same could be true for the use of a low dose of CsA early after transplantation [12]. In this study however, we did not find a difference in the incidence or duration of delayed graft function or in the duration of dialysis between the groups. Nevertheless, some findings indicated a beneficial effect of a lower CsA dose on graft function. Firstly, according to predefined criteria, there were less episodes of CsA nephrotoxicity. Secondly, the number of biopsies was lower in the low CsA group, and this could largely be attributed to a reduction in the number of biopsies showing no abnormalities. As the finding of a histologically normal transplant biopsy in the setting of a rise in creatinine is highly suggestive of acute CsA induced renal dysfunction, it appears that the use of a low dose of CsA can help to avoid this latter condition.

The use of CsA is frequently attended by side effects like hypertension, hyperlipidaemia, neurological symptoms and hirsutism, requiring additional therapy in the majority of renal transplant recipients [13] . The incidence of these side effects could possibly be lowered by reducing the dose of CsA. However, in our study blood pressure, the number of antihypertensive drugs, cholesterol levels, the incidence of adverse events, and the severity of side effects as reported in the patient questionnaire did not differ between both groups.

A possible explanation for the lack of differences in rejection incidence and side effects could be that, although the CsA trough levels for the two groups were different, for individual patients the AUC of CsA may have been highly variable. It is now well known that trough levels and AUC are only weakly correlated, even for the microemulsion preparation [14], and that the total exposure to CsA, represented by the AUC of CsA, has a better correlation with the acute rejection incidence and early graft survival than trough levels of CsA [8;14]. However, repeated measurement of AUC's is very laborious. A promising approach is the monitoring of CsA dose based on mini-AUC's or 2 hour post-dose levels [15]. Possibly, this would have given us a better separation between the two groups and a better chance of finding differences in rejection incidence or CsA-related side effects.

From the start of this study, standard treatment of renal transplant recipients in the participating centers was changed from CsA plus prednisone to triple therapy with CsA, prednisone and MMF. It was our impression that this switch to triple therapy significantly increased CMV - related morbidity, with gastrointestinal localizations being the most prominent finding. In a subsequent retrospective analysis of patients who were at risk for a primary CMV infection, it was found that the addition of MMF did not affect the incidence of CMV infection, but did increase the risk of developing CMV disease [16]. Like many other centers we now routinely use ganciclovir prophylaxis in CMV seronegative patients who receive a graft from a CMV seropositive donor.

By lowering the CsA dose, a significant reduction of $ 500 per patient in the costs of CsA could be obtained. However, the overall effect of the introduction of MMF on the costs of renal transplantation remains uncertain, since the addition of MMF leads to a major increase in the costs of immunosuppressive maintenance therapy. On the other hand, by lowering the rejection rate, substantial savings in the costs of hospitalization and anti T-cell therapy can be achieved. A formal pharmacoeconomic analysis is required to evaluate the cost-benefit balance of the use of MMF more thoroughly [17].

The results obtained in this study could be flattered by the use of a selected study population. However, the three participating centers together perform about half of all renal transplants in The Netherlands, and in each of the centers more than 90% of all eligible patients agreed to participate in the study. Furthermore, several patient categories known to have a higher risk for acute rejection (e.g. second transplantation or the presence of preformed HLA-antibodies) were included in the study. Due to the composition of our general population however, the number of Afro-American patients, known to have a higher risk for acute rejection too [18], was small (< 5%). Taken together, we think that our study group is representative for all our renal transplant recipients, and that the risk of bias by using a selected population is very low.

In summary, we have demonstrated that the addition of MMF to a standard immunosuppressive regimen consisting of CsA and prednisone, allows the use of a lower than usual dose of CsA during the first three months after renal transplantation without increasing the risk of acute rejection. Besides some evidence for a decline in the incidence of CsA nephrotoxicity, the reduction in the CsA dose was not accompanied by an obvious decrease in CsA related side effects. However, savings on drug expenditure would be a major benefit of widespread use of a lower CsA dose.

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Appendix: Questions regarding side effects, caused by the use of CsA, MMF and prednisone. The severity of side effects was scored by the patients on a semiquantitative scale (0=absent; 1= slight; 2= mild; 3=severe).

1. Fatigue
2. Feeling weak
3. Feeling ill
4. Sleep disturbance
5. Increased appetite
6. Feeling obese
7. Increased hair growth
8. Swollen face
9. Acne
10. A dry or itching skin
11. Headache
12. Dizziness
13. Trembling of the hands
14. Paresthaesias of the hands
15. Painful or stiff muscles
16. Dyspnea
17. Edema of the ankles
18. Hematomas
19. Gingival hypertrophy
20. Gingival bleeding
21. Nausea or vomiting
22. Decreased appetite
23. Pyrosis
24. Upper abdominal pain
25. Diarrhoea
26. Bone pain
27. Fractures

Chapter 4.4

Conversion of mycophenolate mofetil, cyclosporine and prednisone to mycophenolate mofetil with or without cyclosporine or prednisone 6 months after kidney transplantation

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Abstract

Background. Triple drug treatment consisting of mycophenolate mofetil (MMF) combined with cyclosporine (CsA) and prednisone (Pred), has become the standard immunosuppressive regimen after kidney transplantation in many centres. Uncertainty exists regarding the necessity of continuing triple drug treatment for more than 6 months after transplantation. We performed a randomised trial, in which CsA or Pred was withdrawn or triple drug treatment was continued at 6 months after renal transplantation.

Methods. In this multi-centre study, renal transplant recipients treated with MMF, CsA, and Pred were randomised at 6 months after transplantation to (1) stop CsA, (2) stop Pred or (3) continue triple drug therapy. Patients with an unstable graft function and patients with 2 or more acute rejections within the first 6 months after transplantation were excluded. The MMF dose was 1000 mg bid and target whole blood trough levels of CsA were 150 ng/ml. The prednisone dose was 0.10 mg/kg/day; in patients discontinuing CsA, prednisone was increased to 0.15 mg/kg/day. Follow-up was continued until 24 months after transplantation.

Results. 212 patients were randomised: 63 in the MMF/Pred group, 76 in the MMF/CsA group and 73 in the MMF/CsA/Pred group. At randomisation, there were no differences between the groups in graft function, CsA dose and level, or rejection incidence before randomisation. Biopsy proven acute rejection occurred in 14/63 patients after CsA withdrawal compared to 3/76 in the Pred withdrawal group (p=0.0014), and compared to 1/73 in the control group (P=0.0001). Graft loss occurred in 2/63 (p=ns) after CsA withdrawal and in 1/76 in the Pred withdrawal group (p=ns), compared to 2/73 in the control group. Biopsy proven chronic rejection was present in 9/63 (p=0.0058) and in 4/76 (p=n.s.) patients in the CsA and Pred withdrawal groups, respectively, compared to 1/73 in the control group. Two patients in the MMF/CsA and 1 patient MMF/CsA/Pred group died with a functioning graft. During follow-up no significant changes occurred in serum creatinine between each of the three groups, although there was a significant increase in serum creatinine for the Pred withdrawal group between the 6 month and 24 month time point. Pred withdrawal resulted in a reduction in mean arterial pressure. Proteinuria was significantly increased at the end of follow-up for both withdrawal groups.

Conclusion. Compared to continuation of MMF/CsA/Pred, CsA withdrawal at 6 months after transplantation results in a significantly increased incidence of biopsy-proven acute and

chronic rejection at 18 months follow-up. Pred withdrawal results in a reduction in mean arterial pressure.

Introduction

The addition of mycophenolate mofetil (MMF) to cyclosporine (CsA) and prednisone results in a decrease in the incidence of acute rejections during the first 6 months after kidney transplantation [1-4]. Whether or not MMF has a positive effect on the development of chronic rejection, either resulting from fewer early acute rejection episodes or as a specific MMF-related effect, is still a matter of debate [4-6]. Meanwhile, long-term continuation of triple drug therapy, in view of the possibility of over-immunosuppression is a cause of concern. Furthermore, both CsA and prednisone have specific drug-related adverse effects on cardiovascular risk factors that may negatively influence long term outcome. Therefore, we performed a randomised, prospective multi-centre study to compare the effect of withdrawing CsA or prednisone from a triple drug regimen consisting of MMF, CsA and prednisone in stable renal transplant patients 6 months after transplantation, with patients continuing triple drug therapy as controls.

Materials and Methods

Between January 1997 and January 2000, 313 patients, transplanted in the university hospitals of Rotterdam, Utrecht and Nijmegen in the Netherlands, entered a study evaluating the cyclosporine sparing effect of MMF in the first 6 months after transplantation[7]. In this study patients were randomised to conventional (target trough level:300 ng/ml) versus low (target trough level: 150 ng/ml) dose CsA treatment during the first 3 months after transplantation in combination with MMF and Pred. From 3 to 6 months after transplantation both groups had the same target trough level of CsA (150 ng/ml). After completion of 6 months follow-up, 212/313 (68%) of these patients agreed to participate and were randomised for this multi-centre, open-label trial of CsA or prednisone withdrawal.
In the first 6 months after transplantation 17 patients had lost their graft and 13 patients died with a functioning graft. Excluded from randomisation were patients with two or more acute rejections during the first six months after transplantation (n=15), patients with biopsy proven

chronic vascular rejection (n=3), patients with proteinuria of more than 3 grams per day (n=2), patients with an unstable graft function (n=9), and patients not treated with triple drug therapy (MMF, CsA, prednisone) at the time of randomisation (n=29). At the time of randomisation 7 patients refused to participate in this study. In 5 patients the treating physician did not ask for participation in this part of the study, because of a bad HLA-mismatch, a previous severe acute rejection or liver function disturbances. One patient was lost to follow-up before randomisation.
The study design was approved by the institutional review boards of the three participating hospitals, and written informed consent was obtained from all participants.

Immunosuppression
Patients were treated with 2 g MMF daily, 0.1 mg/kg prednisone and cyclosporine trough levels targeted between 125-175 ng/ml (from 3 months after transplantation). The micro emulsion formulation of CsA (Neoral$^{(R)}$) was used in all patients. CsA whole blood levels were measured with a monoclonal antibody against the CsA parent molecule, using the FPIA assay on an Abbott TDx analyser (Abbott Laboratories , North Chicago, IL, USA) or with an EMIT assay on an COBAS-MIRA analyser (Dade-Behring, San José, CA, USA). MMF was administered in a fixed dose of 1000 mg bid. Dose reduction or interruption of MMF treatment was allowed in cases of leucocytopenia or anaemia, primary CMV infection or severe gastro-intestinal side effects. The patients randomised for discontinuation of CsA, reduced the CsA dose by 50% for two weeks before complete cessation, whilst increasing the prednisone dose to 0.15 mg/kg and continuing MMF 2 g daily. For 9 patients a 50% CsA dose reduction was accomplished during 4 weeks before complete discontinuation. The patients randomised for discontinuation of prednisone, tapered off the prednisone to 0 mg in 10 weeks according to protocol, whilst continuing CsA and MMF in unchanged dosages. Acute rejections were treated primarily with methylprednisolone (Solumedrol®) 1000 mg iv during three consecutive days. Steroid-resistant rejections were treated with anti T-cell therapy, either rabbit polyclonal antithymocyte globulin (ATG) or a mouse anti-CD3 monoclonal antibody (WT32) [8]. If patients in the withdrawal groups needed anti T-cell rejection treatment, therapy with CsA or prednisone was reinstituted. CMV prophylaxis with ganciclovir or CMV hyperimmune globulin was prescribed during anti T-cell therapy in patients at risk for CMV disease (donor and/or recipient seropositive).

Assessments

At baseline, the medical history, physical examination, routine laboratory tests, lipid profile and histocompatibility data were obtained. Every month vital signs, body weight, and the results of routine laboratory measurements were recorded. Data on rejection episodes, CsA nephrotoxicity, concomitant medication, adverse events, and infections were recorded throughout the entire study period. A biopsy was performed in cases of deteriorating graft function without an obvious pre- or post-renal cause or in cases of increasing proteinuria. No protocol biopsies were performed.

Biopsies were examined by the local pathologist and were classified according to the Banff 1993 biopsy scoring system (grade 1 (=mild), grade 2 (=moderate) and grade 3 (=severe) rejection)[9]. Patients were presumed to have acute rejection if anti-rejection treatment, without prior biopsy, resulted in a decrease in serum creatinine without an obvious pre- or post-renal cause. The creatinine clearance was calculated according to the Cockroft and Gault method. Infections were classified using the CDC definitions for nosocomial infections [10].

Randomisation procedure

Patients were randomly assigned to one of the treatment groups in a 1:1:1 ratio, with stratification for cadaveric / living related transplant, for centre, and for the number of rejections during the first six months after transplantation. Randomisation was carried out by opening a sealed envelope with the lowest available study number.

Statistical analysis

Primary endpoints for analysis were: first biopsy-proven acute rejection after conversion from triple to double immunosuppressive therapy and biopsy-proven chronic rejection during the first 2 years after transplantation. Secondary endpoints were: patient and graft survival, renal function at 1 and 2 years after transplantation, the incidence of infections and malignancies, changes in blood pressure and lipid metabolism.

Analysis performed were based on intention-to-treat. For comparisons within groups a paired non-parametric test was performed (Wilcoxon signed rank test). When appropriate, a student t test was performed. For comparisons within and between different groups a non-parametric ANOVA was performed (Kruskal-Wallis test). Comparison of time to first biopsy-proven rejection was performed using the Kaplan-Meier procedure with Log Rank testing. Multiple logistic regression analysis was performed for determining risk-factors associated with the occurrence of acute rejection after randomisation. The following variables were entered:

previous low or conventional CsA dose, gender, age, PRA, number of transplantation, post-mortal or living donor, HLA mismatches, serum creatinine at randomisation, biopsy-proven rejection during the first 6 months and randomisation group.Results are given as medians with range, unless stated otherwise. A P-value < 0.05 was considered statistically significant. Calculations were performed using the software programs SPSS 8.0 for Windows (SPSS Inc., Chicago, IL, USA), and Graphpad Instat®, version 3.00 for Windows (Graphpad Software Inc., San Diego, CA, USA).

Results

Six months after transplantation, 63 patients were randomised for discontinuation of CsA, 76 patients for discontinuation of prednisone and 73 patients for unchanged continuation of triple therapy. Baseline characteristics are summarised in table 1; no statistically significant differences existed between the groups.

Table 1. Baseline characteristics at time of randomisation, at 6 months after kidney transplantation. There are no statistically significant differences between groups.

Group	MMF/Pred	MMF/CsA	MMF/CsA/Pred
N=patients	63	76	73
Age (yr)	52 (20-72)	52 (19-68)	51 (19-70)
Female/Male	21/42	24/52	27/46
First/Second graft	57/6	68/8	64/9
Cadaveric/Living donor	48/15	58/18	54/19
HLA-A,B,DR Mismatches*	0.78±0.58	0.85±0.62	0.78±0.61
CsA:			
Dose (mg/day)*	283±102	288±85	288±84
Level (ng/ml)*	160±39	158±40	158±42
PRA (>10%):			
Historical (N=)	27	30	35
Recent (N=)	11	10	17
Rejections 6 months (biopsy-proven)	10	11	8

* mean ± standard deviation

Rejections and biopsies:

During the study period a total of 47 renal biopsies were performed, 33 first, 13 second and 1 third biopsy. In the CsA withdrawal group there were 19 first, 9 second and 1 third biopsies. In the Pred withdrawal group there were 9 first and 3 second biopsies. In the control group there were 5 first and 1 second biopsies.

Acute rejections:

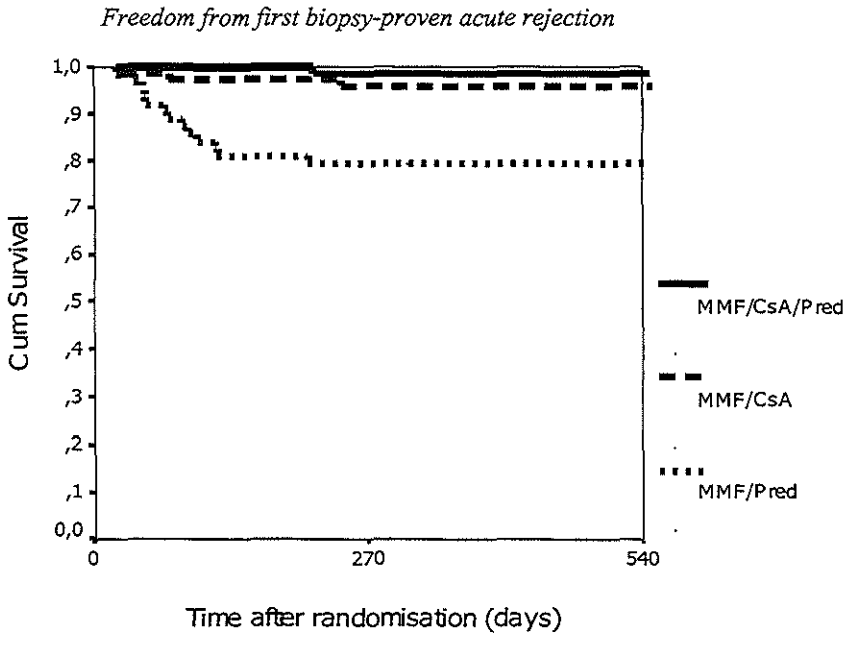
In table 2 all first biopsy-proven acute rejections following randomisation are shown. The time to first biopsy-proven acute rejection is shown in a Kaplan-Meier curve (figure 1).
In a multiple logistic regression model, the only variable that was statistically significantly related to the occurrence of acute rejection after randomisation, was the group for which patients were randomised (p=0.0003).
In the CsA withdrawal group 2 patients had a presumed acute rejection. No biopsy was performed in these 2 patients, but a course of Solumedrol® resulted in recovery of the serum creatinine to initial values. In the CsA withdrawal group 9 patients withdrew CsA during a 4 week period, 1 patient developed a biopsy-proven acute rejection. The occurrence of acute rejection was not significantly different from the patients withdrawing CsA in 2 weeks. A second biopsy-proven acute rejection occurred in 5 patients in the CsA withdrawal group in 2/5 cases this second acute rejection was steroid responsive. Two patients in the CsA withdrawal group had a repeat biopsy within 2 weeks demonstrating ongoing rejection, these patients had already restarted CsA, as had 2 other patients with a 2nd biopsy-proven acute rejection. One patient in the CsA withdrawal group was treated with Solumedrol® for a presumed second rejection.
In the Pred withdrawal group 1 patient developed a second biopsy proven acute rejection, requiring Solumedrol® treatment.
The only patient randomised for triple drug therapy who developed an acute rejection, had stopped MMF because of side effects whereafter the rejection occurred (day 216). The median time to first biopsy-proven rejection was 89 days (22-309) and 74 days (26-260) for the Pred and CsA withdrawal groups, respectively.

Table 2. First biopsy proven acute rejections, according to the BANFF '93 classification, and anti-rejection therapy needed per patient per group.

Group	MMF/Pred	MMF/CsA	MMF/CsA/Pred
N=patients	63	76	73
Acute rejection	14*#	3#	1*
BANFF I	5	2	1
BANFF II	9	1	0
Rejection therapy:			
Solumedrol	13	4	1
Anti-T cell	6	2	0

Comparison between groups: * P=0.0001, # P=0.0014

Fig. 1. Kaplan-Meier survival curve, demonstrating the time to first biopsy-proven acute rejection for all groups from 6 months after transplantation during a follow-up period of 18 months.



Chronic rejections:

In the CsA withdrawal group 9 patients had histological changes compatible with chronic rejection, which was present in 5 patients without concomitant acute rejection.

Four patients in the Pred withdrawal group had histological changes compatible with chronic rejection, 2 patients had concomitant acute rejection.

One patient had histological changes compatible with chronic rejection in the control group.

The overall incidence of chronic rejection in biopsies was 9/63 in the CsA withdrawal group ($p=0.0058$) and in 4/76 Pred withdrawal group ($p=0.0839$), respectively, compared to 1/73 in the control group.

Two patients had histological signs of recurrence of their original kidney disease in their biopsy (CsA and Pred withdrawal group). Histological changes compatible with CsA nephrotoxicity was present in 1 patient in the CsA withdrawal group, in 3 patients in the Pred withdrawal group and in 4 patients in the control group.

In 1 patient in the CsA withdrawal group, and in 3 patients in the Pred withdrawal group the biopsy did not show abnormalities on histological examination.

Graft failure, patient death and treatment failure

Graft failure without patient death occurred in 2/63 patients in the CsA withdrawal group, the cause of graft failure was chronic rejection in both patients. Graft failure, without patient death, occurred once in the Pred withdrawal group because of chronic rejection. In 2 patients in the control group immunosuppressive medication was stopped leading to graft failure. The reasons for cessation of medication were: severe myositis (1 patient), and post-transplant lymphoma, this patient died 2 months hereafter. Patient death with functioning graft only occurred in the groups continuing CsA (3/149). The causes of death were: 1 myocardial infarct, whilst 2 patients were found dead at home, presumably as a result of fatal myocardial infarction (table 3).

Treatment failure, defined as changes in immunosuppressive medication or reinstitution of the discontinued drug, was performed for several reasons (table 4); in the CsA withdrawal group calcineurin inhibitors were mostly restarted after acute rejection episodes needing anti-T cell therapy (protocol driven), prednisone withdrawal was uneventful for almost all patients.

Table 3. Patient and graft survival following conversion per group after 1 ½ year follow-up. Incidence of malignancies per group.

Group	MMF/Pred	MMF/CsA	MMF/CsA/Pred
N=patients	63	76	73
Graft failure	2	1	2
Patient death	0	2	2
Malignancies:	0	1	2
PTLD	0	0	1
Skin (non-melanoma)	0	1	1

At the end of follow-up the daily CsA dose in the Pred withdrawal group was 250 mg (range: 100-500) compared to 250 mg (range:150-550) in the control group (p=0.3446). The CsA trough levels at the end of follow-up were 140 ng/ml (range:60-215) and 138 ng/ml (range:50-330) in the Pred withdrawal group and the control group, respectively (p=0.7791). In the control group MMF dose was reduced to less than the standard 2 grams daily dose in 10/73 compared to 2/63 in the CsA withdrawal group (p=0.035), respectively. This occurred mostly during the latter part of the study by judgement of the physician at the out-patient clinic.

Table 4. Treatment failure per group during 1 ½ year follow-up after conversion.

Group	MMF/Pred	MMF/CsA	MMF/CsA/Pred
N=patients	63	76	73
Medication never stopped	1	1	n.a.
Medication restarted	14‡	4‡	n.a.
Medication stopped:			
MMF	3	0	5
CsA	n.a.	0	1
Pred	0	n.a.	0
All	0	0	1
Total (N=)	18#	5#	7

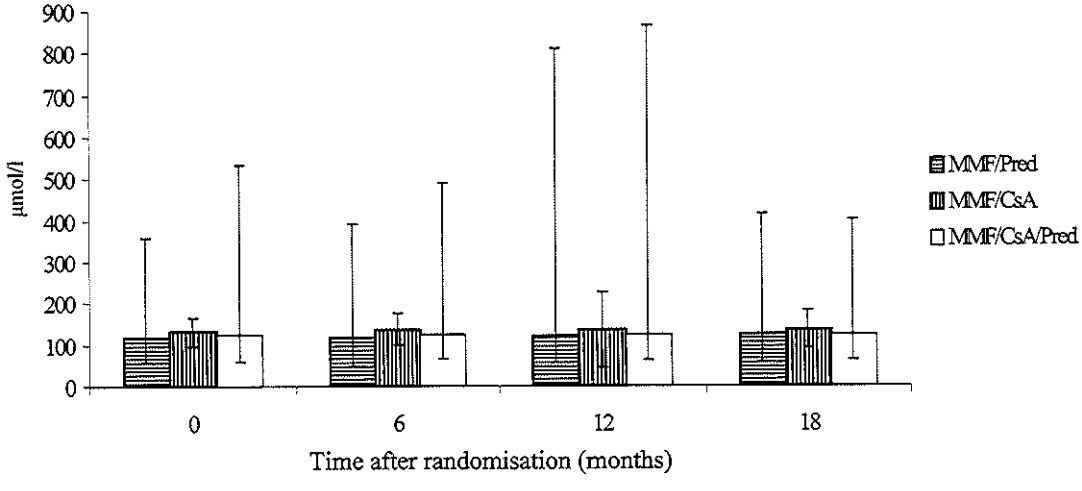
n.a.= not applicable
Comparison between groups: ‡ P= 0.0044, # P= 0.0331

Except for one patient, who completely discontinued MMF, none of these patients experienced an acute rejection following MMF dose reduction or discontinuation. In the Pred withdrawal group in 6/76 patients the MMF dose was reduced.

Graft function at 1 and 2 years after transplantation

There were no statistically significant differences (ANOVA) in serum creatinine between the three groups at 6,12,18 and 24 months (fig 2). Also there were no differences in serum creatinine within the three groups at the various time points (ANOVA). When comparing only the 6 vs 24 month creatinine, a statistically significantly difference ($P<0.05$) for the Pred withdrawal group was present; 130 µmol/l (61-242) vs 137µmol/l (65-293), respectively. The creatinine clearance (Cockroft-Gault), was not statistically significant different within and between all groups at 6, 12 and 24 months (ANOVA). The creatinine clearances in the CsA withdrawal group at 6, 12 and 24 months were: 62 ml/min (30-100), 66 ml/min (31-100) and 64 ml/min (18-104), respectively. The creatinine clearances in the Pred withdrawal group at 6, 12 and 24 months were: 61 ml/min (31-115), 58 ml/min (30-117) and 58 ml/min (28-103), respectively. The creatinine clearances in the control group at 6, 12 and 24 months were: 65 ml/min (20-156), 63 ml/min (22-121) and 65 ml/min (34-116), respectively.

Fig. 2. Serum creatinine (median±range) for all groups from time of randomisation, 6 months after transplantation to the end of follow-up.



At randomisation proteinuria (>0.5 g/day) was present in 18% of patients in the CsA withdrawal group, 16% in the prednisone withdrawal group and in 15% in the control group. Two years after transplantation these percentages were 18% in the CsA withdrawal group compared to 20% in the prednisone withdrawal group (P=ns), and 12% in the control group (P=ns). There were statistically significant differences (non-parametric ANOVA) within groups when comparing proteinuria at 6 months and after 18 months follow-up (table 5).

Table 5. Proteinuria (mean±sd) at time of randomisation and at the end of follow-up

	Month 6	Month 24	P
Proteinuria (g/day)			
MMF/Pred	0.25±0.5	0.57±1.3	<0.01
MMF/CsA	0.22±0.4	0.40±1.0	<0.05
MMF/CsA/Pred	0.26±0.6	0.23±0.3	<0.05
Proteinuria > 0.5 g/day (N=)			
MMF/Pred	11	11	ns
MMF/CsA	12	15	ns
MMF/CsA/Pred	11	9	ns

Between groups the amount of proteinuria at 6 months compared to 24 months was statistically significant different (non-parametric ANOVA) for the Pred withdrawal group compared to the CsA withdrawal group (P<0.01). For the control group the 6 months proteinuria was statistically significant different (non-parametric ANOVA), compared to the 24 months proteinuria in both the CsA (P<0.001) and the Pred (P<0.01) withdrawal groups. Three months after withdrawal of CsA a slight decrease in serum albumin was found: 43±3 g/l compared to 42±3 g/l (P=0.013), respectively. In the Pred withdrawal group the leucocytes decreased significantly 3 months after withdrawal: 7.7±2.6x10^9/l vs 6.3±2.2x10^9/l (P<0.001), respectively. In the MMF/CsA/Pred the hemoglobin increased from 7.9±1.2 to 8.2±1.2 (P<0.001), respectively. There were other no changes in haematological parameters, glucose and albumin 3 months after withdrawal of CsA or Pred, compared to continuation of MMF/CsA/Pred.

Incidence of infections and malignancies:

The incidence of infections, during total follow-up, between all groups was not different: 1.4 infections/patient in the CsA withdrawal group, 1.2 infections/patient in the Pred withdrawal group and 1.3 infections/patient in the control group. Neither was there a difference in the distribution or type of infections between groups (table 6).

Only 3 malignancies occurred during the 18 months follow-up (table 3). One post-transplant lymphoma was diagnosed in the control group. This patient had been treated with r-ATG in the first 6 months after transplantation because of an acute rejection episode. The other 2 malignancies were skin carcinomas (non-melanoma). One patient of these patients had had a previous transplantation.

Table 6. Number of infections for all groups during follow-up (18 months), after randomisation.

Group	MMF/Pred	MMF/CsA	MMF/CsA/Pred	P
N=patients	63	76	73	
Viral infections				n.s.
H.Simplex	5	5	8	
H.Zoster	2	3	0	
CMV	4	1	2	
Opportunistic infections				n.s.
Candida stomatitis	3	3	1	
oesophagitis	0	1	1	
Bacterial infections				n.s.
Urinary tract	36	31	33	
Pneumonia/bronchitis	3/2	3/8	5/10	
Upper respiratory tract	13	16	16	
Skin	7	6	3	
Gastro-intestinal tract	4	3	3	
Other	1	1	4	
Sepsis	3	0	2	n.s.
Culture negative infection	3	12	10	n.s.
Total	85	93	98	n.s.

Cardiovascular risk factors, blood pressure and lipid metabolism

To appreciate the immediate effect of withdrawal of CsA and Pred on the lipid metabolism, comparison at the time of randomisation and 3 months after randomisation is given in table 7. During this 3 month period 1/63 patients in the CsA withdrawal group, 4/73 patients in the Pred withdrawal and 7/73 patients in the control group were treated with cholesterol lowering drugs. The difference in the number of patients treated with cholesterol lowering drugs in the control group compared to the CsA withdrawal group during the first 3 months after randomisation almost reaches significance with a P-value of 0.0681.

Table 7. Effect of withdrawal of CsA or Pred compared to continuation of MMF/CsA/Pred therapy on lipid metabolism at 6 and 9 months (mean±s.d., paired t-test).

	Month 6	Month 9	P
MMF/Pred			
Total Cholesterol (mmol/l)	6.56±1.85	6.03±1.69	<0.001
HDL-Cholesterol (mmol/l)	1.22±0.37	1.25±0.33	n.s.
LDL-Cholesterol (mmol/l)	4.13±1.57	3.95±1.36	n.s.
Ratio total/HDL	5.8±2.6	5.3±2.7	0.0471
MMF/CsA			
Total Cholesterol (mmol/l)	6.42±1.61	6.11±1.25	0.024
HDL-Cholesterol (mmol/l)	1.24±0.52	1.04±0.40	<0.001
LDL-Cholesterol (mmol/l)	4.13±1.29	3.97±1.04	n.s.
Ratio total/HDL	6.0±2.4	6.6±2.8	0.0001
MMF/CsA/Pred			
Total Cholesterol (mmol/l)	6.76±1.66	6.62±1.57	n.s.
HDL-Cholesterol (mmol/l)	1.24±0.39	1.24±0.36	n.s.
LDL-Cholesterol (mmol/l)	4.35±1.44	4.32±1.35	n.s.
Ratio total/HDL	6.0±2.4	5.7±2.2	n.s.

In table 8 all results regarding changes in cardiovascular risk factors at randomisation and at the end of follow-up are shown. Only the prednisone withdrawal group had a statistically significant reduction in mean arterial pressure without a significant increase in the number of

antihypertensive drugs needed. A decrease in total and LDL cholesterol was present for all three groups at the end of follow-up. In all groups physicians were allowed to treat patients with cholesterol lowering drugs. In the CsA withdrawal group 18% of patients used cholesterol lowering drugs, in the prednisone withdrawal group 24% and in the control group 25% at the end of follow-up. These differences in use of cholesterol lowering drugs are not statistically significant.

Table 8. Cardiovascular changes per patient per group; blood pressure, number of anti-hypertensive drugs needed and lipid metabolism at randomisation and after 1 ½ year follow-up after conversion (mean±sem, paired t-test).

	MMF/Pred			MMF/CsA			MMF/CsA/Pred		
	6 months	24 months	P	6 months	24 months	P	6 months	24 months	P
MAP	105±1.4	101±1.5	0.037	104±1.4	101±1.4	0.017	104±1.3	107±2.9	0.413
AHD	1.2±0.12	1.5±0.14	0.027	1.3±0.13	1.4±0.13	0.086	1.3±0.11	1.7±0.13	<0.001
Lipid metabolism									
Total cholesterol *	6.6±0.2	6.2±0.2	0.003	6.4±0.2	5.7±0.2	<0.0001	6.8±0.2	6.2±0.2	0.001
LDL-cholesterol *	4.1±0.2	3.8±0.2	0.001	4.1±0.1	3.6±0.1	<0.0001	4.4±0.2	3.8±0.2	0.003
HDL-cholesterol *	1.2±0.05	1.3±0.08	n.s.	1.2±0.06	1.1±0.07	0.001	1.2±0.05	1.4±0.07	n.s.
Triglycerides *	2.6±0.3	2.2±0.3	0.029	2.6±0.2	2.1±0.2	0.007	2.6±0.3	2.3±0.2	0.054

MAP=Mean arterial pressure (mmHg)

AHD= number of antihypertensive drugs

*=(mmol/l)

Discussion

The combination of MMF, CsA and Pred has decreased the incidence of acute rejection to 20% in the first year after kidney transplantation[1-4]. Long term graft survival, censoring patient death, is primarily influenced by the occurrence of chronic rejection with subsequent development of renal failure [11;12]. Patient survival, death with a functioning graft, is primarily influenced by the high incidence of cardiovascular disease and malignancies in these patients. Renal transplant patients are at an increased risk of myocardial ischemia and infarction when compared with age- and sex-matched healthy controls [13-15]. The incidence of cardiovascular disease in renal transplant patients was calculated to be fivefold greater than predicted from Framingham Heart Study data for patients of comparable age and gender[16]. Furthermore,

maintaining a high immunosupressive load is associated with a higher incidence of malignancies on the long term [16]. Cardiovascular mortality accounts for 16-36% of all deaths in renal transplantation and 9-12% of all deaths are due to malignancy [USRDS 1999]. Unwanted side-effects of both Pred and CsA, such as hyperlipidemia and hypertension, contribute to the increased risk for developing cardiovascular disease [17;18]. No adverse effect on cardiovascular risk-factors of MMF is known. Discontinuation of either CsA or Pred could positively influence the cardiovascular risk-factors, albeit with a small risk of acute rejection [19;20]. The risk of rejection or even graft loss must be weighed against the benefits of stopping either CsA or Pred [14]. Recently, Halloran et al proposed the existence of 2 distinct, probably overlapping, periods after transplantation [21]. The first 6 months, characterised by predominantly immunologically mediated events, the acute rejection phase, probably requires adequate full-dose immunosuppressive drug therapy. In the subsequent maintenance phase a tailor-made immunosuppressive drug regimen may reduce long-term cardiovascular, infectious and malignancy-related morbidity.

The 3-year follow-up from the European MMF study indicated a modest but not statistically significant effect of the addition of MMF on graft survival, which was attributed solely to the lower incidence of acute rejection [5]. Data from the U.S. renal transplant scientific registry demonstrated a reduction in the occurrence of chronic rejection for patients continuing MMF independent of acute rejection compared to patients treated with azathioprine in combination with calcineurin-inhibitors with or without prednisone [22]. As long as data demonstrating the need for continuation of full dose triple drug therapy to achieve superior long-term patient and graft survival are not available, the risks of over-immunosuppression favour discontinuation of one or more drugs. In a recent meta-analysis by Kasiske et al [23], the risk for acute rejection after CsA withdrawal (11%) or Pred withdrawal (14%) was once again outlined. However, despite the association with acute rejection following discontinuation of either drug, only Pred withdrawal was related to increased graft loss during long-term follow-up (>5 years). In our study patients continuing MMF, CsA, Pred had the least acute and biopsy-proven chronic rejections, although there were less biopsies performed in this group. Furthermore, the presence of chronic rejection in biopsies in the CsA withdrawal group, was often diagnosed in the setting of an acute rejection which might over estimate this finding. No statistically significant evidence of over-immunosuppression or increased cardiovascular risk was evident for these patients continuing triple drug therapy, although the follow-up period is too short to

properly assess this. A significantly increased number of patients discontinuing CsA experienced an acute rejection. A large proportion of these acute rejections were of moderate or high severity (grade II in 9/14). Severe vascular acute rejections are known to have a poor prognosis for long-term graft survival [24]. No detrimental effect on creatinine clearance was noted. Prednisone withdrawal resulted in few acute rejection episodes compared to the control group, as was also previously published by Grinyó et al[25]. Prednisone withdrawal was not associated with a significant decrease in creatinine clearance. A decreased clearance, which might result in long-term graft failure, has previously been reported [23]. In our study significantly more proteinuria was present at the end of follow-up for the Pred withdrawal group, which might indicate the presence of chronic rejection.

Although no significant differences exist in patient survival between groups, the causes of death are cardiovascular in patients continuing CsA. The patients in the control group required more anti-hypertensive medication to prevent a rise in mean arterial pressure. In contrast in the Pred withdrawal group there was a significant decrease in mean arterial pressure without the need of more medication. This positive effect of prednisone withdrawal on blood pressure has previously been reported for patients withdrawing prednisone 1 year after kidney transplantation [26]. The Leiden group also published long-term follow-up data (>5 yr) of patients withdrawing CsA from 3 months after transplantation. The patients in whom CsA was withdrawn had less cardiovascular deaths, less hypertension and better renal function compared to patients continuing CsA [27]. The CsA withdrawal group in our study did have a decrease in mean arterial pressure, but this was achieved through more anti-hypertensive medication. As a consequence of the intention-to-treat analysis the effects of discontinuation of CsA are obscured by the proportion of patients in whom this drug was reintroduced (29%). In a conversion study with patients switching from CsA to MMF, beneficial effects on blood pressure and lipid profile were also noted [20].

Do the results of our study help decide which immunosuppressive regimen is the best? The relatively short follow-up of 18 months potentially underestimates the benefits of improving the cardiovascular risk-profile in the withdrawal groups. The number of acute rejections in the CsA withdrawal group could deter clinicians to follow this strategy. There is a clear need for screening tests with which we can identify those patients at increased risk for acute rejection following tapering of immunosuppressive medication. A recently developed test of precursor cytotoxic T-lymphocytes in peripheral blood appeared to allow the identification of a

subgroup of patients in whom tapering of immunosuppression was safe [28;29]. In the future such tests may aid physicians not only in the selection of patients in whom drug treatment can be tapered, but also to what degree the individual patient can be tapered.

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Chapter 5

THERAPEUTIC DRUG MONITORING

Chapter 5.1

Relation of mycophenolic acid trough levels and adverse events in kidney allograft recipients.

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Three large double-blind randomized trials have shown that the addition of mycophenolate mofetil (MMF) to cyclosporine (CsA) and prednisone results in a significant reduction in the rate of biopsy-proven acute rejection during the first 6 months after kidney transplantation [1-3]. Since then, MMF has been widely used in a daily dose of 2 g. Plasma concentrations of the active immunosuppressant mycophenolic acid (MPA), which is formed following oral administration and conversion of MMF, can be monitored [4,5]. Contrary to CsA, data confirming the usefulness of monitoring MPA concentrations or defining a therapeutic window in terms of plasma trough MPA concentrations are not available. In this report, we present the results of monitoring MPA trough levels in relation to adverse events.

Patients and methods

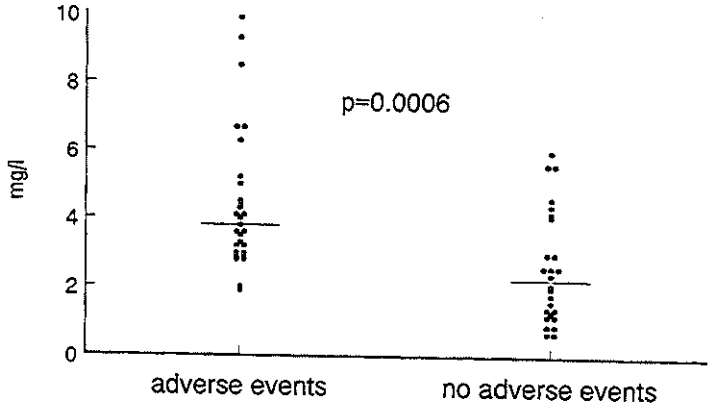
Until 1997 in Rotterdam, renal transplant recipients were treated with CsA and prednisone during the first year after transplantation. Thereafter, patients were converted to azathioprine and prednisone maintenance therapy to improve long-term graft and patient survival [6,7]. In view of the encouraging data obtained with the combination of CsA, MMF and prednisone[1-3] and because MMF appeared to be more efficacious than azathioprine, we decided to convert patients from CsA and prednisone to MMF and prednisone as maintenance therapy 1 year after transplantation. So far 24 patients have been converted to MMF and prednisone for at least 2 weeks. All patients were treated with a daily dose of 2 g MMF. In these patients, fasted MPA trough levels were measured since February 1997 (EMIT-Mycophenolic Acid Assay, Behring Diagnostics Inc., San Jose, Calif).

Results

From 15 patients at least three MPA trough levels measurements were available. No side effects were noted in eight of these patients. The other seven patients suffered from hair loss (n=2), anemia (n=4), or both (n=1) after conversion to MMF.
MPA trough levels ranged from 1.90 to 9.94 μg/ml (mean=4.43±0.38; median 3.79) in patients with side effects and from 0.66 to 6.03μg/ml (mean=2.62±0.32; median 2.26) in patients without side effects (P=0.0006; Mann-Whitney test). All MPA trough levels (n=55) of these 15 patients are shown in Fig 1. When the mean MPA trough levels of each individual

patient are compared, to avoid the fallacy of multiplicity of the units of analysis [8], again a significant difference is found (P=0.02; Mann-Whitney test). None of the 15 patients developed an acute rejection after conversion from CsA to MMF.

Fig.1. MPA trough levels (n=55 samples) in 15 kidney transplant recipients treated with MMF+prednisone showing higher trough levels in patients with adverse events compared to patients without adverse events (P=0.0006, Mann-Whitney test).



Discussion

This paper shows the results of MPA monitoring in kidney transplant recipients on maintenance treatment with MMF and prednisone. In our patients, increased levels of MPA were significantly related to the occurrence of side effects, albeit in a small group of patients. The high incidence of hair loss is remarkable, a side effect which was not found in th three large randomized trials [1-3]. One explanation could be that the hypertrichosis associated with CsA treatment counteracted the MMF-induced hair loss. The hair loss observed was not merely due to the disappearance of the excess hair growth from the period of CsA treatment. In contrast, real alopecia was associated with the use of azathioprine [9]. Another possibility is that MMF toxicity in non-CsA-treated patients is different from that in patients treated with

the combination of CsA and prednisone. In fact we have found clear differences in MPA concentrations between MMF-treated patients with or without CsA. The MPA concentrations in non-CsA-treated patients were significantly higher, possibly due to increased glucuronidation in the CsA group as a result of CsA-induced induction of the cytochrome p-450 complex. Although in kidney transplant recipients a clear reduction in the incidence of acute rejections with MMF has been found [1-3,10], a further improvement of the outcome using therapeutic drug monitoring still has to be shown. This holds true for MPA trough level monitoring in relation to acute rejection as well as to side effects. Whether there is a certain therapeutic window of optimal MPA levels is unclear so far. At present, we feel uncomfortable about patients with relatively high MPA levels, even if these patients do not have overt signs of adverse events, because of the risk of overimmunosuppression. A dose reduction, bringing trough levels to below 4 mg/l, would be our suggested strategy in these circumstances.

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Chapter 5.2

Therapeutic drug monitoring in solid organ transplantation

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Abstract

With the recent introduction of a number of new immunosuppressive drugs there is an unprecedented interest in therapeutic drug monitoring of immunosuppressive drugs. Most published data on therapeutic drug monitoring in transplantation come from retrospective studies, and show a correlation between drug concentrations and toxicity or between concentrations and efficacy. No randomized studies comparing traditional dosing (dose reduction if side effects, dose increase if insufficient efficacy) with concentration-controlled dosing have been done. Nevertheless, for a number of immunosuppressive drugs pharmacokinetic monitoring, based on trough levels, is routine practice. Pharmacodynamic monitoring of immunosuppressive drugs has not reached the stage of widespread clinical application. Prospective investigations on the contribution of therapeutic drug monitoring may result in further improvement of the safety and efficacy of our immunosuppressive regimens and more refined methods for therapeutic drug monitoring.

Introduction

Therapeutic drug monitoring (TDM) is advocated to provide information about the adequacy of a dosing regimen or the likelihood of toxicity associated with a drug. Such situations include: (a) narrow therapeutic interval, meaning toxic symptoms close to concentration with full therapeutic effect; (b) large intra- and interindividual variabilities in pharmacokinetics; (c) presence of factors interacting with drug pharmacokinetics or pharmacodynamics; (d) therapeutic failure on standard dosing; and (e) check for compliance[1]. Current clinical immunosuppressive regimens consist of combinations of drugs, in order to allow a reduction in the individual drug doses as a means to widen the therapeutic interval and to reduce the likelihood of individual drug toxicity. Combining drugs with different mechanisms of action may result in additive or even synergistic effects, potentially allowing dose reduction of individual drugs.

Usually the total immunosuppressive load aimed for in the first weeks or months after transplantation is higher than after a longer interval following transplantation. For a number of drugs, such as cyclosporine (CsA) and tacrolimus (TRL), this is achieved by a reduction in the target plasma concentrations over time. Such ranges have been developed by retrospective

review of drug concentration data and correlation with clinical outcome. For other drugs, such as prednisone, the dose is reduced over time.

Several approaches can be used to assess the appropriateness of the dosing regimen for an immunosuppressive drug[2]. The first, assessment of clinical response, has serious limitations because signs of rejection or toxicity may be difficult to recognize clinically. The second involves assessment of the pharmacokinetic (PK) properties of the drug, such as trough concentration and area under the time-concentration curve (AUC)). The third approach, pharmacodynamic (PD) monitoring, involves measuring the biological effect of the drug at its target site. The most important theoretical advantage of PD monitoring is that it takes into account the inter-individual differences in susceptibility to the drug. Also, the combined use of a number of immunosuppressive durgs could affect the concentration response relationship, making PK monitoring less reliable. Ideally TDM should cost-effectively lead to improved efficacy of the drug and to a reduction in side effects. TDM will be most effective if there is a large interpatient variability and a small intrapatient variability.

For both PK and PD monitoring a reliable analytical technique is crucial. Calibrator accuracy, assay precision and sensitivity and specificity of the assay should be main issues when assessing methodology. Unfortunately the choice of analytical technique often depends upon factors such as the technical facilities available within a laboratory, the target sample turnaround time, cost, and the application for the assay, e.g. drug measurements following chronic therapy or after a single dose[3]. There is a continuing and growing need for external quality assessment of assays to measure immunosuppressive drugs, if the results are to be of any value for other centers.

In this review we will discuss the current status of PK and PD monitoring for azathioprine, cyclosporine, tacrolimus and mycophenolate mofetil.

Azathioprine

Azathioprine - Pharmacokinetic monitoring

The mechanism of action of azathioprine (Aza) is not fully understood. After the conversion of Aza to 6-mercaptopurine (6-MP), an intracellular metabolism to thioguanine nucleotides occurs which, through their antiproliferative effect on lymphocytes, sets off the cytotoxic, and

probably also the immunosuppressive effects of the drug. It is unclear what the contribution is of the 6-MP metabolites, 6-thioinosinic acid and 6-thioguanylic acid. Interpatient variability in Aza pharmacokinetics is substantial, with an up to 50-fold variation in dose-adjusted Aza AUC[4]. Pharmacokinetic monitoring is not being done, because of the difficulties measuring Aza and 6-mercaptopurine, and because of uncertainties as to which compound or metabolite is related to the effect or toxicity.

Azathioprine - Pharmacodynamic monitoring

Monitoring the effect of Aza can be done by measurement of red blood cell 6-thioguanine nucleotides. The formation of 6-thioguanine nucleotides from 6-mercaptopurine is catalysed by thiopurine methyltransferase (TPMT). TMPT activity is determined by an allelic polymorphism for either high or low enzyme activity[5]. Homozygotes for the low activity allele are known to be at risk for profound myelosuppression with Aza. On the other hand, homozygotes for the high activity allele may be inadequately immunosuppressed with conventional, empiric doses of Aza. Determination of the TMPT activity in erythrocytes of transplanted patients could be a good predictor of the hematological risk of Aza treatment[6]. However, this approach is not being used by most clinicians. Current practice is to start with a fixed dose (based on body weight) and reduce dose on the basis of adverse events such as leucopenia or anaemia.

Cyclosporine

Cyclosporine - Pharmacokinetic monitoring

Cyclosporine is metabolized by the cytochrome p450 system (CYP3A), which is present not only in the liver, but also in intestinal epithelium and renal tubular epithelium. The low oral bioavailability of cyclosporine is not primarily an absorption problem. Extensive metabolism in the intestinal epithelial cells, p-glycoprotein efflux and hepatic metabolism are the major determinants of the substantial presystemic elimination[7,8]. Although some of the interindividual variability in first-pass metabolism can be accounted for by genetic factors, the remainder appears to primarily reflect modulation of CYP3A activity by environmental

factors[9]. CYP3A appears to be particularly susceptible to induction and inhibition by many compounds. It is therefore no surprise that there is no consistent relationship between dose and blood concentrations. Whole blood measurements are preferable to those made on plasma or serum as distribution between red cells and the extracellular compartment in vitro varies with storage of the sample and time before separation[10].

The first study suggesting a relationship between cyclosporine concentration and immunosuppressive activity in renal transplant recipients was reported by Keown in 1981[11]. Later, for cyclosporine-related side-effects, a strong correlation with drug levels was found[12]. Although both the efficacy and the toxicity of cyclosporine show a good correlation with blood concentrations of the drug there is no controlled trial demonstrating improved outcome as a result of therapeutic drug monitoring. Most centers have nevertheless adopted the strategy to monitor trough blood concentrations and adjust the drug dose in order to reach a certain predefined target range, the limits of which may differ depending on the organ transplanted, the interval since transplantation and the co-medication. The clinical utility of this approach suffers from the fact that trough concentrations do not reliably predict total drug exposure over 12 or 24 hours at the individual level. Although with the new microemulsion formulation bioavailability of cyclosporine is increased and between-patient variability reduced, even this formulation suffers from the inadequate correlation between trough concentration and drug exposure[13]. A potential risk of using trough level monitoring is that high cyclosporine exposure may go unnoticed, resulting in long term toxicity. The absence of a correlation between cyclosporine trough levels and the incidence of cyclosporine induced nephrotoxicity in heart transplant recipients could be explained by this phenomenon[14,15]. Some groups have therefore advocated the use of abbreviated AUCs in stead of trough concentrations[16,17]. In stable heart transplant patients a greater clinical benefit was observed when Neoral dose monitoring was performed according to a 2-hour sample, compared with trough levels[18]. Measuring drug concentrations other than trough does however require a considerable effort to reliably draw blood at exactly the correct time point. Because of the practical limitations of this approach most centers have not changed their policy of performing TDM on the basis of trough levels yet.

Cyclosporine - Pharmacodynamic monitoring

Several attempts have been made to evaluate the effect of cyclosporine on immune response, rather than by measuring cyclosporine concentration in blood. In cyclosporine treated multiple sclerosis patients expression of the Interleukin-2 receptor (IL-2R) and the production of IL-2 were reduced after in vitro stimulation of the patients' peripheral blood mononuclear cells by PHA, compared with untreated patients.

A more direct pharmacodynamic parameter of cyclosporine activity is the measurement of the inhibition of calcineurin[19]. Calcineurin is the target of inhibition by cyclosporine and tacrolimus in T-lymphocytes[20], and its activity can be monitored in peripheral blood[21]. Further studies are required to determine whether calcineurin activity is correlated with efficacy or adverse events.

Tacrolimus

Tacrolimus - Pharmacokinetic monitoring

Tacrolimus displays a considerable inter-individual variability in absorption and clearance[22]. Oral bioavailability has been reported to range from 4% to 89%, irrespective of the organ transplanted[23]. Twelve hour trough concentrations are recommended for routine TDM post-transplantation. Compared to cyclosporine there is a better correlation between trough levels and AUC for tacrolimus[24]. Whole blood is the preferred matrix for monitoring tacrolimus levels[25].

The rationale for monitoring comes from the observation of a relationship between tacrolimus concentration and toxicity in transplant patients[26]. There are no studies demonstrating a reduced incidence of acute rejection based on TDM.

Like cyclosporine, tacrolimus is a substrate for the cytochrome p450 enzyme system, more specifically the CYP 3A isoform subfamily[27]. A genetic polymorphism for this enzyme system has been found, and partly explains the inter-individual variability in metabolic rate for these drugs[28]. There would be considerable cost associated with screening all patients for genotype before starting treatment with either of these drugs. Moreover, the inter- and intra-individual variability in pharmacokinetics of cyclosporine and tacrolimus is more due to

environmental factors than to the CYP 3A polymorphism. Therefore, current practice remains dosing based on trough levels.

Tacrolimus - Pharmacodynamic monitoring

In the early years of tacrolimus use in clinical trials Zeevi et al developed a bioassay[29]. They studied the inhibitory effect of tacrolimus on lymphocyte proliferation and compared the results of their bioassay with the tacrolimus plasma concentrations measured with ELISA in liver transplant recipients. Tacrolimus levels determined with the bioassay were lower than those measured by ELISA, of which at that time it was not known what the specificity for biologically non-active metabolites was. A similar experience was reported for a biopsy growth assay in heart transplant patients[30].
Like cyclosporine, tacrolimus mediates its immunosuppressive activity trough inhibition of calcineurin. Monitoring the activity of calcineurin therefore would be a means to pharmacodynamically monitor tacrolimus treatment. Data correlating tacrolimus concentrations, calcineurin activity and clinical outcome are required to determine the usefulness of this approach.

Mycophenolate mofetil

Mycophenolate mofetil – Pharmacokinetic monitoring

Following studies with mycophenolic acid in rats and non-human primates[31,32] it was shown that the addition of mycophenolate mofetil to a cyclosporine-based immunosuppressive regimen significantly reduces the incidence of acute rejection after kidney transplantation[33]. In this respect MMF has been shown to be superior to azathioprine[34,35]. A randomized trial of MMF in renal transplant patients treated with a tacrolimus-based regimen showed a similar significant reduction in the incidence of rejection in the first year after transplantation[36].
The randomized concentration-controlled trial has shown a significant correlation between mycophenolic acid-AUC (MPA-AUC) and the incidence of acute rejection after kidney transplantation[37]. A similar correlation between drug concentrations and efficacy was made by Oellerich et al in a pediatric kidney transplant population[38].

Clinical data from our center show that MPA trough levels in kidney recipients treated with MMF and prednisone are significantly higher than from patients on MMF, cyclosporine and prednisone[39,40]. There is a considerable inter-patient variability in the pharmacokinetics for MPA.

The studies that led to the registration of MMF for use in solid organ transplantation all used cyclosporine-based immunosuppressive regimens[33-35]. As an increasing number of new immunosuppressive drugs now become available, MMF will be used more and more in non-cyclosporine containing treatments. Our data show that this will have an important influence on exposure to MPA, as MPA-AUC will be substantially higher without the use of cyclosporine[39,40]. That this will lead to more side-effects is already shown by Shapiro et al[36]. In regimens where, after combined treatment with MMF and cyclosporine for the first 6 or 12 post-operative months, the latter is stopped clinicians must realize that this will consequently lead to a rise in MPA concentrations.

The first suggestion of a therapeutic window for MPA was formulated in 1998 by a consensus panel and recommended to be a MPA-AUC of 20 μg.hr/mL or more, in the early post-transplant period[41]. Based on studies in which renal and heart transplant recipients were treated with cyclosporine, MMF and corticosteroids Shaw et al have defined a proposal for therapeutic drug monitoring. They propose to estimate a patient's MPA-AUC using an abbreviated AUC in the first 2 weeks after surgery and aim for a target between 30 and 60 μg.hr/mL42 . From their experience in pediatric renal transplant recipients Oellerich et al have defined exactly the same lower and upper limit for MPA-AUC[38]. Renal insufficiency decreases protein binding of mycophenolic acid and increases free fraction, both by a direct effect of the ureamic state and by competition of the retained metabolite MPAG[43]. Both Shaw and Oellerich stress the importance of free fraction and suggest other targets for patients with low albumin levels or renal failure[38,42,44]. By performing MMF dose adjustment based on MPA trough level monitoring Meiser et al have significantly reduced the incidence of acute rejection after heart transplantation in a small cohort of patients[45].

Mycophenolate mofetil – Pharmacodynamic monitoring

Mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil (MMF), inhibits inosine monophosphate dehydrogenase (IMPDH), a key enzyme for the de novo purine

biosynthesis[46,47]. Therefore, to date the concept of MPA's mechanism of immunsuppressive action has been limited to its suppression of alloantigen-stimulated T and B cell proliferation[32,48]. Pharmacodynamic monitoring of MMF measures IMPDH activity. IMPDH activity measurements have been compared with drug level monitoring in rabbit[49] and human[50] blood. There are however no data that show a good correlation between MMF induced inhibition of IMPDH activity and drug efficacy or the incidence of MMF related side effects.

At Stanford University new methods for assessing the immunosuppressive effects of MPA on lymphocytes after in vivo treatment were developed in order to investigate new mechanisms of the immunosuppressive action of MPA by determining whether MPA inhibits processes in immune cells in addition to its known inhibition of lymphocyte proliferation. Studies in heart transplanted rats showed that administration of different dose levels of MPA not only suppressed the proliferation of lymphocytes in the blood of Lewis rats, but also suppressed the expression of CD25 and CD134[51,52] . In these transplantation studies pharmacodynamic effects correlated highly with MPA area-under-the-plasma-concentration time curve (AUC) and with MPA concentrations 6 hours after dosing. These pharmacodynamic values also correlated well with rejection scores. In view of the strong correlation between pharmacokinetic and pharmacodynamic data, these studies support further investigations in using pharmacodynamic monitoring for therapeutic drug monitoring of MMF treatment.

Conclusions

With the recent introduction of mycophenolate mofetil and tacrolimus there is an unprecedented interest in therapeutic drug monitoring of immunosuppressive drugs. It is remarkable that therapeutic drug monitoring has gained so much interest. Most published data are from retrospective studies, and show a correlation between drug concentrations and toxicity and between concentrations and efficacy. Studies showing the value of therapeutic drug monitoring with a proper study design are not available. No randomized studies comparing traditional dosing (dose reduction if side effects, dose increase if insufficient efficacy) with concentration-controlled dosing have been done. At present for cyclosporine or tacrolimus this kind of study would probably be judged to be unethical, but for mycophenolate mofetil there still is a chance to do so.

Pharmacodynamic monitoring of immunosuppressive drugs has not reached the stage of widespread clinical application. In part this is caused by the fact that most of the pharmacodynamic assays are time-consuming, costly and in some cases only give a result after several days of incubation. Another reason for the limited interest in pharmacodynamic monitoring is the lack of data showing improved outcome if dose adjustment is based on pharmacodynamics rather than pharmacokinetics. On the other hand, such data are also lacking for pharmacokinetic monitoring.

Methodological requirements are high, to ensure acceptable accuracy and reproducibility. Internal quality control and continuing proficiency testing are needed for between-centre comparisons. For most, if not all, drugs the non-protein bound fraction is considered to be the pharmacologically active component of the total amount of drug present. The unbound fraction can vary considerably without affecting the total blood concentrations. Measurement of free fraction however is complicated and not an option for routine monitoring in most centers at the present time.

It is remarkable that pharmacokinetic monitoring is being performed on such a large scale, while scientifically sound data showing improved outcome as a result of therapeutic drug monitoring are scarce[53]. Multicenter concentration-controlled clinical trials can provide a basis for designing future prospective TDM investigations[54]. Defining the best set of concentrations of the combinations of immunosuppressive drugs may show further improvement of the safety and efficacy of our immunosuppressive regimens.

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Chapter 6

SUMMARY AND CONCLUSIONS

Summary and conclusions

Summary

This thesis describes pharmacokinetic studies on mycophenolate mofetil in renal transplant recipients. Furthermore, we describe the efficacy and safety of mycophenolate mofetil in combination with various immunosuppressive schedules both in the first 6 months after kidney transplantation and in the maintenance period hereafter. The goal of these clinical studies was to find the optimal immunosuppressive drug therapy for the kidney transplant recipient, for whom patient survival is predominantly influenced by cardiovascular disease and graft survival by chronic allograft rejection.

In **chapter 1** an introduction to the mechanism of action and the use of mycophenolate mofetil for several, predominantly auto-immune, diseases is described.

In **chapter 2** the aims and outline of the thesis are described.

In **chapter 3** pharmacokinetic studies on mycophenolic acid trough levels, the active compound of the pro-drug mycophenolate mofetil, in the presence or absence of cyclosporine are described. A previously unknown influence of cyclosporine on mycophenolic acid trough levels in kidney transplant recipients who discontinued cyclosporine was first recognised in Rotterdam. Although extensive pharmacokinetic studies on mycophenolate mofetil had been performed before its use in kidney transplantation, it was always done in combination with cyclosporine. In a cross-sectional study we noted that patients who were treated with mycophenolate mofetil and prednisone, cyclosporine free, had higher mycophenolic acid trough levels compared to patients treated with cyclosporine, mycophenolate mofetil and prednisone. In a prospective randomised study patients were randomised to continue cyclosporine, mycophenolate mofetil and prednisone or discontinue either cyclosporine or prednisone at 6 months after transplantation. This study convincingly confirmed these findings, with an almost doubling of mycophenolic acid trough levels in the patients discontinuing cyclosporine compared to the other 2 patient-groups. We evaluated the relation

of mycophenolate mofetil dose and mycophenolic acid trough levels in a cyclosporine free protocol and found a significant relation between both.

In **chapter 4** strategies regarding the optimal use of mycophenolate mofetil have been studied. The first study (retrospective) describes the results of withdrawing mycophenolate mofetil at least 6 months after transplantation, whilst continuing cyclosporine and prednisone. Patients in this study were among the first to receive mycophenolate mofetil in Rotterdam and Nijmegen, before its official registration in the Netherlands and even before the results of the three 'pivotal' trials were published. Because of the risk of over-immunosuppression and uncertainty about the added advantage of continuing mycophenolate mofetil for long term graft and patient survival, mycophenolate mofetil was tapered and stopped. In these stable renal transplant recipients no acute rejections occurred, demonstrating the safety of withdrawal of MMF.

The second study (prospective, randomised) focussed on the safety of conversion from cyclosporine to either mycophenolate mofetil or azathioprine in stable renal transplant recipients 1 year after transplantation. In order to avoid cyclosporine related nephrotoxicity and increase in cardiovascular risk factors conversion to azathioprine has been common practice in our centre for several years, before this study. Because of superior efficacy of mycophenolate mofetil compared to azathioprine if combined with cyclosporine and prednisone as demonstrated in the three pivotal trials, we decided to perform a study comparing mycophenolate mofetil with azathioprine in the absence of cyclosporine. Indeed, significantly less acute rejections occurred after conversion to mycophenolate mofetil compared to azathioprine, with the added advantage of a better renal function after discontinuing cyclosporine in both groups. To decrease the overall immunosuppressive load, which limits the increased susceptibilty to infections and malignancies, dose reductions were performed at 4 and 8 months after conversion. Apart from conversion related acute rejections, did three patients experience an acute rejection when mycophenolate mofetil was given in a total daily dose of 1 g in combination with prednisone, as did three patients treated with 1 mg/kg azathioprine. A clear correlation was found between mycophenolic acid trough levels and adverse events, no correlation was found for the occurrence of acute rejection and trough

levels. The majority of patients (72%) could be converted and tapered in their immunosuppressive medication.

The third clinical study (multi-centre, prospective, randomised) demonstrates the cyclosporine-sparing potential of mycophenolate mofetil during the first 6 months after transplantation, the time-period in which most acute rejections occur. Two groups of patients were treated with mycophenolate mofetil, prednisone and cyclosporine targeted at two different trough levels the first 3 months. In the control group cyclosporine was targeted at conventional cyclosporine trough levels and the treatment group had target levels at 50% of the control group. Despite lower cyclosporine trough levels, no increase in the incidence of acute rejections was found. Furthermore, no difference in renal function, blood pressure, lipid metabolism or infectious complications was found. The reduced amount of cyclosporine gave a cost saving of $500 per patient.

The fourth clinical study (multi-centre, prospective, randomised) examines the necessity of continuing triple drug therapy as maintenance treatment, with the potential risk of over-immunosuppression and the added negative impact of continuation of cyclosporine and prednisone on cardiovascular risk factors. This study is a continuation of the cohort of patients from the third clinical study. After 6 months patients with a stable graft function were randomised to either continue mycophenolate mofetil, cyclosporine and prednisone or discontinue cyclosporine or discontinue prednisone. The patients in whom cyclosporine was withdrawn experienced significantly more acute and chronic rejection compared to the other patient groups, but did not result in more graft or patient loss during a 18 month follow-up period. There was a positive effect on the mean arterial pressure for the patients discontinuing prednisone, as well as on the lipid metabolism. However, these patients discontinuing prednisone had a significant increase in their serum creatinine at the end of the follow-up period. The amount of proteinuria increased significantly for the patients in the cyclosporine and prednison withdrawal group at the end of follow-up. There were no significant differences in the incidence of malignancies, infections or patient survival.

In **chapter 5** the role of therapeutic drug monitoring in organ transplantation is discussed. Therapeutic drug monitoring is widely advocated worldwide for most immunosuppressants used, although prospective, randomised studies comparing outcome between fixed, rational

dosing versus drug monitored dosing have not been performed. The current status of both pharmacokinetic and pharmacodynamic monitoring for azathioprine, cyclosporine, tacrolimus and mycophenolate mofetil are discussed. One randomised concentration controlled trial in adults and one study in pediatric renal transplant recipients showed a significant correlation between mycophenolic acid area-under-the time curve and the incidence of acute rejection after kidney transplantation.

Conclusions

From the studies performed several observations regarding the optimal use of mycophenolate in combination with other immunosuppressive drugs can be made. The efficacy of mycophenolate mofetil in combination with prednisone and cyclosporine targeted at low trough levels of 150 ng/ml in the prevention of acute rejection during the first 6 months after renal transplantation is clearly demonstrated. This study confirms that mycophenolate mofetil has a cyclosporine sparing effect. Discontinuing cyclosporine 6 months after transplantation exposes ±20% of these patient to an acute rejection with a significant increase in biopsy-proven chronic rejection. Prednisone withdrawal increases the amount of proteinuria as does cyclosporine withdrawal after 18 months follow-up. Discontinuing mycophenolate mofetil at least 6 months after transplantation does not seem to increase the risk of acute rejection, but does not reduce the potential long term influence of cyclosporine on cardiovacsular risk factors. Conversion of cyclosporine to mycophenolate mofetil at 1 year after transplantation seems a good option with a renal sparing effect, dose reduction to 750 mg bid appears to be safe. When discontinuing cyclosporine, when used in combination with mycophenolate mofetil, a substantial rise in mycophenolic acid levels can be expected, with a resulting increase in adverse effects. Dose reductions can be performed guided by trough level measurements, although no prospective data regarding the optimal mycophenolic acid trough level are present related to the occurrence of acute rejection.

We conclude that:

1. Cyclosporine decreases mycophenolic acid trough levels

2. Mycophenolate mofetil withdrawal is safe from 6 months after kidney transplantation, when combined with cyclosporine and prednison.
3. Mycophenolate mofetil has a cyclosporine sparing effect
4. Conversion from cyclosporine to mycophenolate mofetil 1 year after kidney transplantation and dose reduction to 750 mg twice daily is safe. Dose reduction to 500 mg twice daily induces the risk of acute rejection
5. Prednisone withdrawal is safe, but results in a significant increase in proteinuria
6. Mycophenolic acid trough levels are related to adverse events

Chapter 7

SAMENVATTING EN CONCLUSIES

Samenvatting en conclusies

Samenvatting

In dit proefschrift worden studies beschreven betreffende de farmacokinetiek van mycofenolaat mofetil bij niertransplantatie patiënten. Daarnaast beschrijven wij de effectiviteit en veiligheid van mycofenolaat mofetil in combinatie met diverse immunosuppressieve schema's , zowel tijdens de eerste 6 maanden na niertransplantatie als tijdens de onderhoudsfase hierna. Het doel van deze klinische studies was om de optimale immuunsuppressieve therapie voor de niertransplantatie patiënt te vinden, wiens overleving voornamelijk beïnvloed wordt door hart en vaatziekten en wiens lange termijn transplantaat overleving beïnvloed wordt door chronische transplantaat afstoting.

In **hoofdstuk 1** wordt als inleiding voor dit proefschrift een overzicht gegeven van het werkingmechanisme en gebruik van mycofenolaat mofetil bij diverse ziektebeelden, waaronder een groot aantal auto-immuun ziekten.

In **hoofdstuk 2** worden de doelstellingen van dit proefschrift beschreven.

In **hoofdstuk 3** beschrijven wij farmacokinetische studies over mycofenolzuur dalspiegels, de actieve component van het pro-drug mycofenolaat mofetil, in aan- en afwezigheid van cyclosporine. Een voorheen onbekende interactie van cyclosporine op mycofenolzuur dal spiegels bij niertransplantatie patiënten die cyclosporine staakten werd voor het eerst ontdekt in Rotterdam. Alhoewel uitvoerige farmacokinetische onderzoeken met mycofenolaat mofetil verricht waren voordat dit medicijn werd gebruikt bij niertransplantaties, was dit altijd verricht in combinatie met cyclosporine. In een cross-sectionele studie vonden wij dat patiënten die behandeld werden met mycofenolaat mofetil en prednison, dus zonder cyclosporine, hogere mycofenolzuur dalspiegels hadden in vergelijking met patiënten die werden behandeld met cyclosporine, mycofenolaat mofetil en prednison. In een prospectieve, gerandomiseerde studie werden patiënten gerandomiseerd om of door te gaan met cyclosporine, mycofenolaat mofetil en prednison, danwel om cyclosprine of prednisone te staken 6 maanden na transplantatie. Deze studie bevestigde op overtuigende wijze de eerder gevonden bevindingen, met bijna een

verdubbeling van mycofenolzuur dalspiegels bij patiënten die cyclosporine staakten in vergelijking met de twee andere patiënten groepen. Wij onderzochten de relatie tussen mycofenolaat mofetil dosis en mycofenolzuur dalspiegels in een "cyclosporine-vrij" protocol en vonden dat er een significante relatie tussen beide bestond.

In **hoofdstuk 4** beschrijven wij strategieën met betrekking tot het optimaal gebruik van mycofenolaat mofetil. In het eerste onderzoek (retrospectief), onderzochten wij de resultaten van het staken van mycofenolaat mofetil, tenminste 6 maanden na transplantatie, terwijl cyclosporine en prednison werden gecontinueerd. Patiënten die aan deze studie participeerden waren de eersten in Rotterdam en Nijmegen die mycofenolaat mofetil gebruikten, vóór de officiële registratie in Nederland en zelfs voordat de resultaten van de drie grote "pivital" studies waren gepubliceerd. Vanwege het risico op over-immunosuppressie en onzekerheid betreffende de toegevoegde waarde van mycofenolaat mofetil voor de lange termijn transplantaat en patiënt overleving, werd mycofenolaat mofetil verminderd in dosering en gestaakt.
Het tweede onderzoek (prospectief, gerandomiseerd) spitste zich toe op de veiligheid van het omschakelen van cyclosporine naar mycofenolaat mofetil of azathioprine bij stabiele niertransplantatie patiënten 1 jaar na transplantatie. Vanwege de mogelijkheid van aan cyclosporine gerelateerde nefrotoxiciteit en tevens het verhoogd risico op hart en vaatziekten werden in ons centrum, voorafgaande aan dit onderzoek, patiënten routinematig gedurende vele jaren van cyclosporine naar azathioprine omgezet. Aangezien mycofenolaat mofetil een superieure effectiviteit heeft in vergelijking met azathioprine indien het wordt gecombineerd met cyclosporine en prednison, zoals aangetoond in de drie grote "pivotal" studies, besloten wij een studie te verrichten waarbij beide medicamenten werden vergeleken in de afwezigheid van cyclosporine. Significant minder acute afstotingen traden op bij de patiënten die werden geconverteerd naar mycofenolaat mofetil in vergelijking met azathioprine, met als toegevoegde waarde een betere nierfunctie na het staken van cyclosporine voor beide groepen.
Om de totale immunosuppressieve druk, die gerelateerd is aan een verhoogde gevoeligheid voor infecties en maligniteiten, te verminderen werden dosis reducties 4 en 8 maanden na conversie verricht. Afgezien van rejecties gerelateerd aan conversie, traden acute afstotingen na dosis reductie bij 3 patiënten op in beide groepen bij een dagelijkse dosis van 1 g mycophenolaat mofetil of 1 mg/kg azathioprine, beide in combinatie met prednison. Er bestond een duidelijke

correlatie tussen mycofenolzuur dalspiegels en bijwerkingen, deze correlatie werd niet gevonden tussen mycofenolzuur dalspiegels en acute afstoting. De meerderheid van de patiënten (72%) kon geconverteerd worden en in hun dosis worden verminderd.

De derde klinische studie (multi-centrum, prospectief, gerandomiseerd) toont het cyclosporine sparende effect van mycofenolaat mofetil aan tijdens de eerste 6 maanden na transplantatie, de tijdepisode waarin de meeste acute afstotingen optreden. Twee groepen patiënten werden behandeld met mycofenolaat mofetil, prednison en cyclosporine gedoseerd om twee verschillende dalspiegels te bereiken gedurende de eerste 3 maanden na transplantatie. De controle groep had conventionele cyclosporine dalspiegels terwijl de onderzoeksgroep cyclosporine dalspiegels had die 50% lager waren dan de controle groep. Ondanks lagere cyclosporine dalspiegels, werd er geen verhoogde acute rejectie incidentie gevonden. Verder was er geen verschil in nierfunctie, bloeddruk, vetmetabolisme of infectieuze complicaties. De verminderde hoeveelheid cyclosporine leverde een kostenbesparing van 1200,- gulden per patiënt op.

De vierde klinische studie (multi-centrum, prospectief, gerandomiseerd) bestudeerde de noodzaak om drie immuunsuppressieve medicamenten te continueren als onderhoudsbehandeling, met het potentiële risico van over-immunosuppressie en de hiermee geassocieerde invloed van zowel cyclosporine als prednison op cardiovasculaire risicofactoren. Deze studie was een vervolg van een cohort patiënten uit de derde klinische studie. Zes maanden na transplantatie werden patienten met een stabiele nierfunctie gerandomiseerd om mycofenolaat mofetil, cyclosporine en prednison te continueren of om cyclosporine of prednison te staken. De patiënten die cyclosporine staakten hadden significant meer acute en chronische rejectie in vergelijking met patiënten uit de andere twee groepen. Dit resulteerde niet in meer transplantaat of patiënten verlies tijdens 18 maanden follow-up. Bij patiënten die prednison staakten werd een verbetering van de gemiddelde arteriële bloeddruk en een beter vetmetabolisme gezien. Daarentegen hadden deze patiënten een significante stijging van hun hoeveelheid proteïnurie aan het einde van de follow-up periode. De hoeveelheid proteïnurie steeg significant voor de patiënten die cyclosporine en prednison staakten aan het einde van de follow-up periode. Er waren geen significante verschillen in de incidentie van maligniteiten, infecties of patiënt overleving.

In **hoofdstuk 5** wordt de rol van therapeutisch drug monitoring besproken. Drug monitoring wordt wereldwijd aanbevolen voor de meeste immuunsuppressieve medicatie die wordt gebruikt, alhoewel prospectief, gerandomiseerde studies die uitkomst (rejectie) tussen vaste, rationeel bepaalde doseringen versus dalspiegel gemonitorde doseringen niet zijn verricht. De huidige stand van zaken met betrekking tot farmacokinetische en farmacodynamische monitoring voor azathioprine, cyclosporine, tacrolimus en mycofenolaat mofetil worden besproken. Een gerandomiseerde concentratie-gecontroleerde studie bij volwassen en een studie bij pediatrische niertransplantatie patiënten toonden een significante correlatie tussen mycofenolzuur oppervlakte-onder-de-curve en de incidentie van acute afstoting na niertransplantatie aan.

Conclusies

Naar aanleiding van alle beschreven onderzoeken met betrekking tot het optimaal gebruik van mycofenolaat mofetil in combinatie met andere immuunsuppressiva kunnen een aantal conclusies worden getrokken. De effectiviteit van mycofenolaat mofetil gecombineerd met prednison en cyclosporine getitreerd op lage dalspiegels van 150 ng/ml ter preventie van acute afstoting tijdens de eerste 6 maanden na niertransplantatie is duidelijk aangetoond. Hiermee bewijst mycofenolaat mofetil een cyclosporine sparend effect te hebben. Het staken van cyclosporine 6 maanden na transplantatie stelt ± 20% van deze patiënten bloot aan een acute afstoting met een significante toename in biopsie bewezen chronische rejectie. Het staken van cyclosporine of prednison verhoogt de hoevelheid proteïnurie na 18 maanden follow-up. Het staken van mycofenolaat mofetil tenminste 6 maanden na transplantatie lijkt zonder verhoogd risico op acute afstoting gepaard te gaan, het continueren van cyclosporine geeft geen vermindering van cardiovasculaire risicofactoren die hieraan zijn gerelateerd. Conversie van cyclosporine naar mycofenolaat mofetil 1 jaar na transplantatie is een goede optie met een nierfunctie sparend effect, en dosis reductie tot 750 mg twee maal daags lijkt veilig. Indien cyclosporine wordt gestaakt bij gelijktijdig gebruik van mycofenolaat mofetil dan kan een aanzienlijke stijging van mycofenolzuur spiegels worden verwacht, met een hieraan gerelateerde toename van bijwerkingen. Dosis reducties kunnen worden verricht met dalspiegel metingen ter controle, alhoewel prospectieve onderzoeken op dit moment ontbreken met betrekking tot de optimale mycofenolzuur dalspiegel gerelateerd aan het optreden van acute afstoting.

Wij concluderen dat:

1. Cyclosporine mycofenolzuur dalspiegels verlaagt
2. Het staken van mycofenolaat mofetil 6 maanden na niertransplantatie veilig is in aanwezigheid van cyclosporine en prednison
3. Mycofenolaat mofetil een cyclosporine sparend effect heeft
4. Conversie van cyclosporine naar mycofenolaat mofetil 1 jaar na niertransplantatie en dosis reductie naar 750 mg twee maal daags veilig is. Dosisreductie naar twee maal daags 500 mg mycofenolaat mofetil de kans op acute rejectie introduceert
5. Het staken van prednison veilig is, maar resulteert in een significante toename van proteïnurie
6. Mycofenolzuur dalspiegels gerelateerd zijn aan het optreden van bijwerkingen

Curriculum Vitae

De auteur van dit proefschrift werd op 23 april 1963 geboren te Dordrecht. Na het behalen van het HAVO en VWO-diploma aan de Scholengemeenschap Louise de Coligny te Leiden startte hij in 1984 met de studie geneeskunde aan de Rijksuniversiteit Leiden. Op 23-08-1991 behaalde hij het artsexamen (cum laude). Van 1991 tot 1993 was hij werkzaam als AGNIO cardiologie en later als AGNIO inwendige geneeskunde in het St. Clara ziekenhuis te Rotterdam. In 1993 begon hij zijn opleiding tot internist, de eerste twee jaar in het Merwede Ziekenhuis te Dordrecht (opleiders: Dr. B.A. de Planque en Dr. J.Ph.H.B. Sybesma), de volgende twee jaar in het St. Clara Ziekenhuis te Rotterdam (opleider: Dr. A.F. Grootendorst) en de laatste twee jaar in het Academisch Ziekenhuis te Rotterdam (opleider: Prof.Dr. M.A.D.H. Schalekamp). In januari 1999 begon hij met de specialisatie in het aandachtsgebied nefrologie (opleider: Prof.Dr. W. Weimar) en sinds 1 juli 2000 is hij als staflid verbonden aan de afdeling inwendige geneeskunde van het Academisch Ziekenhuis Rotterdam/Dijkzigt, sectie transplantatie. Gedurende de opleiding tot internist werd gestart met het in dit proefschrift beschreven onderzoek. Voor een presentatie over het klinische onderzoek beschreven in hoofstuk 4.4 ontving hij in 2001 in Chicago een Young Investigator Award van de American Society of Transplantation.
De auteur is getrouwd met Bettina Samrén en samen hebben zij drie kinderen: Anna, Thomas en Stefan.

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