# NEPHROLOGY
# EXIT EXAMINATION

# Title

## Use of Mycophenolate Mofetil in Renal Transplantation

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# Content

## Abstract

Mycophenolate mofetil (MMF) is the morpholinoethyl ester prodrug of mycophenolic acid (MPA), an uncompetitive reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH activity is the rate-limiting step in the de novo synthesis of guanosine nucleotides, a pathway essential for DNA synthesis in lymphocytes. Lymphocytes rely on de novo pathway and replicating lymphocytes are thus arrested in S-phase by MMF. MMF is generally added to post-transplant therapy regimens in conjunction with cyclosporin and corticosteroids.

There were large randomised controlled trials showing that MMF can significantly reduce the incidence of acute rejection by 50% at 6 months. As acute rejection has a negative impact on long-term graft and patient survival, patients treated with MMF tend to have better outcome at 3 years. In addition to be a prophylaxis for acute rejection, MMF has been shown to be effective in treatment of acute rejection and chronic allograft nephropathy. Lastly, but not least, MMF can be used as a cyclosporin-sparing or corticosteroid-sparing agent. However, MMF has several side-effects. The major ones are gastrointestinal toxicity, haematological toxicity and increased incidence of opportunistic infections.

Therapeutic drug monitoring is necessary for MMF. Evidences show that there was a correlation between the incidence of acute rejection and MPA pharmacokinetics. Since there is interpatient MPA pharmacokinetic variability, individualised MMF dose evaluation is mandatory to optimise its efficacy. Both pharmacokinetic and pharmacodynamic approaches are available for therapeutic drug monitoring.

## Introduction

Mycophenolate mofetil (MMF) is the synthetic morpholinoethyl ester prodrug of mycophenolic acid (MPA), a natural fermentation product of several Penicillium species. Mycophenolate mofetil acts as an antimetabolite immunosuppressant and is generally added to post-transplant therapy regimens in place of azathioprine, and in conjunction with cyclosporin and corticosteroids. It has been registered for use in prevention of allograft rejection in renal transplantation, and is being trialed for the treatment of acute and chronic rejection of renal allograft.

This article will review several aspects:

i) the pharmacokinetic and pharmacodynamic properties of MMF;
ii) therapeutic drug monitoring;
iii) the clinical use of MMF in renal transplantation;
iv) adverse effects of MMF.

## Pharmacodynamic Properties

Mycophenolate mofetil is the morpholinoethyl ester prodrug of mycophenolic acid (MPA), an uncompetitive reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH activity is the rate-limiting step in the de novo synthesis of guanosine nucleotides, a pathway essential for DNA synthesis in lymphocytes. Lymphocytes rely on de novo pathway and replicating lymphocytes are thus arrested in S-phase. Other cells are able to recycle purine bases via a salvage pathway, and thus are not critically affected by MPA activity.

Depletion of deoxyguanosine triphosphate (dGTP) by MPA is pivotal to the inhibition of DNA synthesis, whereas depletion of guanosine triphosphate (GTP) may affect other processes. Maintenance of intracellular GTP stores is essential for the activation of both fucose and mannose to their respective nucleotide sugars, which in turn provide pools for the formation of cellular glycoproteins, including adhesion molecules. MPA can deplete lymphocyte GTP pools and inhibit protein glycosylation, thus affecting the ability of lymphocytes to attach to and invade allograft endothelia [1]. Altered expression of adhesion molecules can also inhibit the recruitment of lymphocytes to sites of inflammation, thus blocking ongoing rejection after clonal expansion has occurred.

## Pharmacokinetic Properties

### (a) Absorption and distribution

After oral administration, MMF is rapidly and completely converted to the active metabolite MPA and the parent drug MMF is not detected in plasma. The mean bioavailability of MPA is 94%. MPA undergoes enterohepatic recirculation, as indicated by a secondary peak in plasma concentrations 6 to 12 hours after administration. The volume of distribution of MPA was about 4L/kg after oral or intravenous administration of MMF. At therapeutic doses, MPA is 97% bound to plasma albumin.

### (b) Metabolism and Elimination

The active metabolite MPA is primarily metabolised in the liver to mycophenolic acid glucuronide (MPAG), the major urinary excretion product. Urinary excretion of MPA is negligible. MPAG is generally regarded as inactive. There are other MPA metabolites that can be identified, including the MPA glycoside conjugate M-1 and the acyl glucoronide conjugate M-2, the latter of which is active against IMPDH.

MPAG is predominantly excreted via the kidneys. It is also secreted in bile but glucoronidases from gut bacteria convert it back to MPA, which is reabsorbed and recirculated.

The apparent elimination half-life of MPA after administration of single-dose oral or intravenous mycophenolate mofetil 1.5 gm to 12 healthy volunteers was 17.9 or 16.6 hours, with a clearance of 11.6 or 10.6 L/hour [2].

### (c) Pharmacokinetics-Efficacy Relationship and Therapeutic Monitoring

Although the current labeling information for MMF does not indicate any need for therapeutic monitoring of plasma MPA concentrations, there were a number of studies showing a relationship between MPA pharmacokinetics and clinical outcome. There is substantial interpatient pharmacokinetic variability for MPA, as is the case for cyclosporin A and tacrolimus. The 12

hour dose interval MPA Area Under Curve (AUC) shows a more than 10 fold range for renal transplant patients on fixed MMF dose of 2 g/day [3]. Thus therapeutic drug monitoring seems to be useful in order to maximise its efficacy.

A retrospective statistical evaluation of MPA dose-interval AUC data in relation to the incidence of acute rejection was performed in patients enrolled in a MMF Japanese renal transplant clinical trial [4]. The study patients were randomized to one of several doses of MMF. They were in addition receiving cyclosporin A and prednisone. There was a significant correlation ($p < 0.001$) between the risk for rejection (relative to the risk with no MMF) and the natural log of the dose-interval MPA AUC, but not to MMF dose.

In addition to the Japanese study, there was also a randomised, double-blind concentration-controlled study of MMF in 156 renal transplant recipients [5]. The hypothesis was to look for any correlation between MPA AUC and acute rejection rate. The patients were randomized to 3 groups designated as low, intermediate or high AUC for MPA; dosage was adjusted to provide target AUC after each measurement. Logistic regression analysis showed a significant ($p < 0.001$) relationship between MPA AUC and the likelihood of rejection. High MPA values were associated with a very low probability of rejection. An AUC of 15 μg·h/ml yielded 50% of maximal achievable efficacy with a 4% change of efficacy for a 1 μg·h/ml change in AUC at the mid-point of the logistic curve. Other variables (e.g. the maximum observed plasma concentration, predose plasma concentration, and drug dose) were found to have poorer predictive power for the rejection outcome.

As determination of AUC is laborious and costly, some researchers tried to investigate if there was a correlation between MPA trough concentration and the likelihood of acute rejection [6]. Data from 48 renal transplant recipients showed that patients with acute rejection ≤ 2 months after transplantation had significantly lower MPA trough level than those without

rejection (1.55 vs 2.1 mg/L, $p < 0.005$) [7]. These data suggest that the mean MPA plasma level should be achieved within a range from 1 to 3 μg/ml to prevent acute rejection in the early period after renal transplantation.

Based on the above results, on-going trials are attempting to determine the most effective means of monitoring plasma MPA profile and to evaluate whether the therapeutic monitoring will provide real benefits with respect to immunosuppression and adverse events. As monitoring over the full 12-hour dose interval to determine $AUC_{0\text{-}12h}$ is laborious and costly, some researchers tried to develope limited sampling strategies for determination of MPA AUC in paediatric kidney recipients. They found wide inter- and intraindividual pharmacokinetic variations in paediatric transplant recipients despite a standardized dosage and thus therapeutic drug monitoring is necessary. They found that three-point limited sampling strategies (based on 0 min, 75 minutes and 6 hours post dose) gives a close correlation ($r^2 = 0.88$) between the full $AUC_{0\text{-}12hr}$ and abbreviated AUC.

At Princess Margaret Hospital, we performed a pharmacokinetic study of MMF in Chinese renal transplant recipients.

**Study Design**

This is a 12-month perspective open-label and single center design. 10 renal transplant patients in our hospital were enrolled in 1998. They were receiving standard doses of cyclosporin A and prednisolone according to the protocol of our center. They were receiving MMF 1 gm BD in the first six months post transplant and then 750 mg BD afterwards.

Pharmacokinetic profiles were measured at the end of first week, first month, third month and one year. For each profile study, blood samples were taken at 0 (trough), 20, 40, 60, 75, 90 minutes and 2, 4, 6, 8, 10, 12 hours and MPA levels were measured by high performance liquid chromatography (HPLC).

## Results

The pharmacokinetic parameters were summarised in Table-1. There were no significant differences in Cmax and Tmax of MPA among different time periods. MPA trough level significantly increased at 1 year compared with 1 week. AUC increased in 1, 3 months and 1 year compared to 1 week post transplant. By multiple regression analysis, abbreviated AUC by 4 time points (0, 1, 2, 4 hr) of MPA has been shown to have reasonable good correlation to actual $AUC_{0\text{-}12hr}$ ($r^2 = 0.89$). The model equation was $6.02 + 5.61 * C_{0h} + 1.28 * C_{1h} + 0.9 * C_{2h} + 2.54 * C_{4h}$.

## Conclusion

MPA AUC increased with time (up to 1 year of study) when compared to one week after renal transplantation. It is thus possible to reduce MMF dosage in late post transplant period to reduce the cost and avoid over-immunosuppression. Abbreviated AUC (0, 1, 2, 4 hr) is a less expensive and more convenient may to represent the MPA exposure.

## Therapeutic Drug Monitoring

Therapeutic drug monitoring of MMF can be either pharmacokinetic or pharmacodynamic monitoring. Pharmacokinetic monitoring is direct measurement of plasma MPA concentration whereas pharmacodynamic monitoring is measurement of the biological effect of the drug. Both of them will be discussed in this section.

### (a) Pharmacokinetic Monitoring

Pharmacokinetic monitoring of MMF can be done by measuring the plasma MPA concentration, either by high performance liquid chromatography (HPLC) or immunoassay technique EMIT.

HPLC method was used for MPA concentration measurement in many pharmacokinetic clinical studies. This method has been implemented successfully in at least 6 laboratories in North America and Europe. Its performance characteristics has been described elsewhere [8-9].

An MPA EMIT immunoassay is under evaluation. It has been described in several reports with acceptable precision [7, 9-13]. The principle feature of EMIT is that the activity of the enzyme label in the antibody-antigen enzyme complex is either inhibited or stimulated, compared with the activity in the free antigen-enzyme conjugate. The catalytic activity of the enzyme measured in this mixture is then either directly or inversely proportional to the antigen content of the sample. The MPA in the sample competes with MPA-labeled enzyme, glucose-6-phosphate dehydrogenase, allowing the MPA level to be measured indirectly.

When compared with HPLC, EMIT is simpler and not so labour consuming. In addition, there were studies showing that EMIT has the advantage of measuring the active metabolites of MPA. Schütz E groups [14] observed a positive bias between the MPA EMIT immunoassay and HPLC and they presumed that crossreactive metabolites of MPA may be present in the

plasma of organ transplant recipients under immunosuppression with MMF. Through HPLC, they could identify two putative metabolites that showed an almost identical UV spectrum to either MPA (M-2) or MPAG. One of these metabolites (M-2) was also immunoreactive in the EMIT assay, suggesting that it may be a major cause for the discrepancies observed between EMIT and HPLC. They further found out that the MPA metabolite M-2 can also inhibit IMPDH activity. As a result, the MPA concentration determined by EMIT will theoretically better reflect immunosuppression by MMF treatment than will values obtained with methods that only measure MPA.

At University of Colorado Health Sciences Center, we tried to evaluate the EMIT mycophenolic acid immunoassay regarding its analytical performances in clinical plasma samples from renal transplant recipients treated with MMF. In addition, we tried to investigate the pharmacokinetic profiles of mycophenolate mofetil in patients taking the same dose of mycophenolate mofetil.

**Patients and Methods**

A total of 87 renal transplant patients were randomly chosen. Their immunosuppressive agents included cyclosporin A, prednisolone and mycophenolate mofetil (twice daily dose). They were advised to take their medication at 10am and 10pm exactly. Blood samples were taken before oral administration of MMF as 12- hour trough level. Drug monitoring was performed by the EMIT-Mycophenolic Acid Assay. The test was performed on a COBAS-MIRA analyser (Roche Laboratory).

**Reagents**

(i) EMIT Mycophenolic Acid Antibody Reagent A: mouse monoclonal antibody reactive to mycophenolic acid; nicotinamide adenine dinucleotide; ≤ 0.1% sodium azide.

(ii) EMIT Mycophenolic Acid Enzyme Reagent B: mycophenolic acid conjugated to glucose-6-phosphate dehydrogenase; ≤ 0.1% sodium azide.

(iii) EMIT Mycophenolic Acid Calibrators: mycophenolic acid in synthetic matrix; ≤0.1% sodium azide. Concentrations (μg/ml): 0, 0.5, 2.0, 5.0, 10, 15.

(iv) EMIT Mycophenolic Acid Controls: mycophenolic acid in synthetic matrix; ≤ 0.1% sodium azide

**Principle**

The EMIT Mycophenolic Acid Assay employs a homogenous enzyme immunoassay technique used for the analysis of MPA in plasma. It is based on competition for MPA antibody binding sites. MPA in the sample competes with MPA-labeled enzyme glucose-6-phosphate dehydrogenase (G6PDH) in Enzyme Reagent B. Active (unbound) enzyme converts the oxidized nicotinamide adenine dinucleotide (NAD) in Antibody Reagent A to NADH, resulting in a kinetic absorbance change that can be measured spectrophotometrically. Enzyme activity decreases upon binding to the antibody, allowing the MPA concentration in the sample to be measured in terms of enzyme activity. Endogenous serum G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme employed in the assay.

**Results**

The mean age of the patients was 46 ± 13 years old. The male : female ratio was 53 : 34. Their mean creatinine level was 1.8 ± 0.7 ug/ml. They were taking MMF at daily doses of 500 mg (n = 3), 1000 mg (n = 7), 1500 mg (n = 7), 2000 mg (n = 66) and 4000 mg (n = 4).

Within-run precision was determined by assaying each of the three controls (low, medium, and high) in replicates of 20 over 3 consecutive days. Between-run precision was evaluated by assaying each of the three controls in replicates of 2 over 10 consecutive days.

The coefficients of variation (CVs) for both within-run precision and

between-run precision were less than 10%. The limit of detection for the EMIT assay was 0.5 μg/ml.

The mean MPA levels for each dosage groups were summarised in Table-2. There was great variability in MPA levels for each dosage group. The coefficients of variation varied from 34.51% to 81.98%.

**Discussion**

MMF is a potent immunosuppressant which can reduce the incidence of acute rejection and it is routinely used in fixed dose of 2 g or 3 g per day[15-17]. This is different from the standard of practice to measure immunosuppressive drug concentrations (e.g. Cyclosporin A and Tacrolimus) as a guidance of dosage adjustment to protect patient after transplantation either from the risk of rejection or over-immunosuppression.

Our findings showed that there was great inter-individual variability of pharmacokinetic profiles. This is similar to those previously reported by Bullingham RES [3]. As there is more and more evidences suggesting that there is a correlation between pharmacokinetic profile and risk for acute rejection, we propose that therapeutic drug monitoring of MMF is valuable for the following reasons:

(a) to establish the adequacy of MPA concentrations early after transplant surgery;

(b) to provide a basis for how flexible the transplant physicians can be in MMF dose reduction to avoid side-effects; and

(c) to establish a baseline for determination of doses reduction of concomitant immunosuppressive drugs for maintenance immunosuppression.

EMIT assay is one of the methods for measuring the MPA level. It is simpler and not so labour-consuming when compared with HPLC. Further

validation of measuring MPA trough level by EMIT is necessary to establish our own therapeutic window for MPA.

**(b) Pharmacodynamic Monitoring**

Pharmacodynamic monitoring of the biological effect of immunosuppressive drug provides an alternative to traditional therapeutic drug monitoring. This approach has significant advantages over the measurement of drug concentrations, especially in multiple drug therapy, where assessment of the appropriate therapeutic range may be difficult.

Since MPA inhibits IMPDH, pharmacodynamic monitoring of the degree of inhibition of the enzyme may provide a better indicator of immunosuppression than will the drug concentrations in plasma. Longman LJ et al described the method of measuring IMPDH activity in whole blood [18]. Briefly, the enzyme activity is determined by estimating the $^{3}H$ released from [2, 8-$^{3}H$] IMP that has been formed in the cells from added [2, 8-$^{3}H$] hypoxanthine (Hx, 15 Ci/mol) or [2, 8-$^{3}H$] inosine (Ino, 30 kCi/mol) from Moravek Pharmaceuticals, Brea, CA. During the reaction, the tritium atom located on C-2 of the hypoxanthine ring of IMP is replaced by a hydroxy group. $NAD^{+}$ serves as the electron acceptor and is reduced to NADH. The rate of catalysis with the $^{3}H$ substrate is less than that with non-labeled substrate because of the tritium isotope effect.

At University of Colorado Health Sciences Center, we tried to perform simultaneous pharmacokinetic and pharmacodynamic monitoring of MPA in transplant recipients and investigate the correlation of MPA level and IMPDH activity.

**Patients and Method**

A 26-year-old female suffered from Epstein's syndrome and she presented with renal impairment and bone marrow failure. She was put on haemodialysis and she had an allogeneic bone marrow transplantation done at

our hospital. MMF was included in the immunosuppressive regimen. Both pharmacokinetic and pharmacodynamic monitoring of MPA was performed for her and then the dose of MMF was adjusted according to the profile.

For the pharmacokinetic profile, several blood samples were taken pre-dose and at different times post-dose. The MPA level was measured by EMIT MPA assay as described before. The area under curve (AUC) was calculated for each profile.

The blood samples were simultaneously sent for pharmacodynamic measurement of IMPDH activity. For the measurement of IMPDH activity in whole blood, 100 μL (10 μ Ci) of radiolabeled Hx was added into 300 μL of whole blood. After 30 minutes, 100 μL aliquots was removed and mixed with 500 μL of freshly prepared cold suspension of 100 g/L activated charcoal in 50 g/L trichloracetic acid. The samples were then centrifuged at 1300 g for 10 minutes and 200 μL of the supernate was analysed for radioactivity by scintillation counting. The amount of the spontaneous liberation of $^{3}H$ measured with each analysis was small and so this background value was subtracted from all measurements to obtain net values for the amount of $^{3}H$ released enzymatically by IMPDH. The enzyme activity was expressed as counts (disintegrations) per minute (cpm/min).

**Results**

The pharmacokinetic profiles were summaried in Table-3. Following administration of either PO or IV MMF, the plasma profiles showed that there was a rapid rise to achieve peak values at about 1 hour post dose. Such findings were compatible with the study of RES Bullingham R [2].

The correlation between MPA levels and IMPDH activity were summarised in Table-4, Table-5, Fig 1 and Fig 2. We showed that there was an inverse relationship between the MPA level and the IMPDH activity. The maximum inhibition of IMPDH activity was achieved by the peak MPA level.

**Discussion**

MMF is a potent immunosuppressant drug and it acts by reversibly inhibiting the IMPDH. Based on such mechanism, measurement of IMPDH activity seems to be one of the ways to monitor the therapeutic effect of MMF. We have shown that there was an inverse relationship between MPA level and IMPDH activity. IMPDH activity was a good reflection of the MPA level.

Measurement of IMPDH activity is a pharmacodynamic monitoring of the biological effect of MMF. Such pharmacodynamic monitoring may have significant advantage over the traditional monitoring of drug concentrations, especially in multiple drug therapy, where assessment of the therapeutic range may be difficult.

## Clinical Efficacy

MMF can be used as a component of either maintenance immunosuppression for the prevention of acute organ allograft rejection or as rescue therapy for the treatment of acute rejection. Its effect on chronic rejection is also discussed in this section.

### (a) Prevention of Acute Rejection

There were three randomised, double-blind, multicentre trials, involving nearly 1500 adult renal transplant patients, being conducted to evaluate MMF as part of immunosuppressive therapy regimen for the prevention of acute allograft rejection [15-17]. These studies were conducted in the US (14 centres), Europe (20 centres) and Australia, Canada and Europe (Tricontinental Study; 21 centres). All of the studies included cyclosporin and corticosteroids in their immunosuppressive protocols, and the US study included induction therapy with anti-thymocyte globulin. The US and Tricontinental Studies compared MMF with azathioprine while the European Study compared MMF with placebo. All patients received cadaveric renal transplants, either first only (US Study), or first or second (European and Tricontinental Studies). The primary efficacy end-point for each of the studies was biopsy-proven acute rejection or treatment failure, defined as graft loss, patient death or premature withdrawal form the study for any reason within 6 months after transplantation.

The above studies demonstrated that administration of MMF in conjunction with cyclosporin and corticosteroids results in a significant reduction in the incidence of biopsy-proven acute rejection or treatment failure at 6 months compared with azathioprine or placebo in an otherwise identical immunosuppressive regimen. Biopsy-proven acute rejection alone was reduced by about 50% in MMF group (13.8 to 19.8%) compared with azathioprine group (35.5 to 38.0%), and by up to 70% compared with placebo group (46.4%). At 6 months, graft loss or death (with or without prior rejection) was not significantly different across the treatment groups in any of

these studies. The primary cause of graft loss in all groups was rejection; there was a trend towards a reduced incidence of graft loss due to rejection in the MMF group, which was significant when the data were pooled (see following subsection). Withdrawals due to adverse events accounted for the majority of other treatment failures in the MMF groups.

The European placebo-controlled study reported results for the primary end-point in patients at 1 year after transplantation [19]. The incidence of biopsy-proven rejection or treatment failure remained significantly lower in MMF group when compared with the placebo group. The number of first biopsy-proven rejection episodes did not increase significantly in any treatment group after the first 6 months in this study. Graft loss or death at 1 year after transplantation was similar across all patient groups.

The 3 multi-centre studies were designed so that their results could be integrated into pooled efficacy analysis after 1 year of patient follow-up in order to assess graft and patient survival, incidence and treatment of acute rejection episodes and graft function at 1 year [20]. The combined analysis showed that the proportion of patients with a first acute rejection episode at 1 year was significantly different among the treatment groups. The relative risks for at least one episode of biopsy-proven acute rejection were 0.46 for MMF 2 g/day and 0.38 for MMF 3 g/day versus placebo/azathioprine ($p < 0.0001$). MMF could also decrease the severity of rejection episodes [21].

Acute rejection occurred most commonly in the first 3 months of treatment and was the leading cause of graft loss at one year. The relative risks of graft loss due to rejection was about 0.40 and 0.54 for MMF 2 g/day and MMF 3 g/day respectively. However, MMF did not have significant effect on overall graft loss. Patient deaths were equally distributed between the treatment groups.

When compared with the placebo/azathioprine group, MMF group had a significantly lower rate of biopsy-proven rejection or treatment failure at 1

year. The effect was very similar to the data at 6 months. This suggests that MMF did not simply delay acute rejection, but prevented it as long as MMF treatment was maintained.

All 3 studies have been carried out to 3 years of post-transplant follow-up for analysis of graft loss and patient death [22-24]. Despite the studies were not powered to demonstrate a significant difference for these end-points, there was a consistent trend towards improved outcomes over 3 years for patients treated with MMF 2 or 3 g/day compared with the azathioprine or placebo group.

The three year data support that early acute rejection has a negative impact on long term graft and patient survival. Graft loss or death was around 4 times more prevalent in patients who experienced a biopsy-proven acute rejection episode within the first 6 months after transplantation (26 to 40%) than those with no rejection episodes during this period (5.7 to 10%). In the Tricontinental Study, MMF 3 g/day produce a better outcome than MMF 2 g/day beyond the 6-month time point. In the other 2 studies, MMF 2 g/day had a more favourable risk: benefit ratio than did MMF 3 g/day. As a result, the recommended dosage for use in renal transplantation is 2 g/day.

Akinlolu et al recently presented their findings of beneficial effect of MMF on late allograft function [25]. They analysed the date of 66,774 renal transplant recipients from the U.S. renal transplant scientific registry. Patients who received a solitary renal transplant between October 1, 1988 and June 30, 1997 were studied. They found that MMF decreased the relative risk for development of chronic allograft failure (CAF) by 27% (RR 0.73, $p < 0.001$). This effect was independent of its outcome on acute rejection. Censored graft survival using MMF versus azathioprine was significantly improved by Kaplan-Meier analysis at 4 years (85.6% vs 81.9%). Thus MMF therapy decreases the risk of developing CAF. This improvement is only partly caused by the decrease of acute rejection observed with MMF; but, is also caused by an effect independent of acute rejection.

At Princess Margaret Hospital, we had performed a study to investigate the efficacy and safety of MMF in Chinese renal allograft recipients [26].

**Materials and Methods**

From 1997 to 1998, 69 primary renal allograft recipients (male: 43 patients and female: 26 patients) were enrolled into either MMF or azathioprine group. All of them were treated simultaneously with cyclosporin A (Neoral) and prednisolone. The initial and maintenance dose of Neoral were 10 mg/kg/day and 3-5 mg/kg/day respectively. The initial and maintenance dose of prednisolone were 30 mg/day and 0.1-0.2 mg/kg/day respectively.

41 patients were enrolled in the MMF group. They were taking 3 different dosages of MMF. 18 patients were taking 1 gm BD. 14 patients were taking 750 mg BD. 9 patients were taking 500 mg BD. The remaining 28 patients were receiving azathioprine 1-2 mg/kg/day.

**Results**

There were no significant differences in sex, age, body weight and primary renal disease between MMF group and azathioprine group. MMF significantly reduced the incidence of acute rejection as compared with azathioprine (14.6 vs 32.1%; $p < 0.05$). Among 3 different dosage groups, there was no significant difference in the incidence of acute rejection (1 gm BD: 16.7%; 750 mg BD: 14.3%; 500 mg BD: 11.1%). Patient survival at 6 months was 100% in MMF group versus 93% in azathioprine ($p = NS$). Graft survival at 6 months was 97% in MMF group versus 93% in azathioprine group ($p = NS$).

**Conclusion**

Our result was comparable to the Western studies and MMF was found to be effective in reducing the incidence of acute rejection in first 6 months by 55% as compared with azathioprine, in conjunction with cyclosporin and

prednisolone. However, there were no significant differences in both patient and graft survival between MMF group and azathioprine group.

**(b) Treatment of Acute Rejection**

In addition to the ability of mycophenolate mofetil to prevent the development of acute rejection, MMF is also shown to reverse rejection when administered as rescue therapy during an established rejection episode in both animal studies and clinical trials. Such effect may be due to its potential to inhibit the glycosylation of adhesion molecules, resulting in restricted recruitment of lymphocytes into sites of inflammation and graft rejection.

***First Acute Rejection***

The Mycophenolate Mofetil Acute Renal Rejection Study Group performed a double-blind, double dummy controlled clinical trial of 221 renal transplant recipients experiencing the first biopsy-proven rejection within 6 months of transplant performed at 15 US and Canadian centers [27]. A total of 113 patients received MMF (1.5 gm BD) and intravenous corticosteroids, and 108 patients received azathioprine (1-2 mg/kg/day) and intravenous corticosteroids. The intravenous corticosteroids in each group consisted of 5 mg/kg/day for 5 days followed by an oral steroid taper. End points for the study were the first use of antilymphocyte therapy, the number of courses of antirejection therapy given during the first 6 months, and graft and patient survival at 1 year.

At 6 months, 16.8% of MMF-treated patients required at least one course of antilymphocyte therapy versus 41.7% of the azathioprine-treated patients ($p < 0.0001$). The number of patients requiring full courses of antirejection therapy for the treatment of rejection was less in the MMF-treated group (24.8%) versus the azathioprine-treated group (58.3%) ($p < 0.0001$). The proportion of patients with the use of antilymphocyte therapy or treatment failure during the first 6 months was 29.2% versus 51.9% ($p = 0.0006$) in the MMF versus the azathioprine groups respectively. After one year, 8.9% of

patients in the MMF group lost their graft or died versus 14.8% in the azathioprine group.

MMF administered in combination with pulse corticosteroids can significantly decrease the subsequent use of antilymphocyte therapy in the treatment of acute renal allograft rejection. MMF together with steroid also improves the rate of reversal of acute rejection episodes.

This study was continued in a nonblind manner after the first year for 3 years of follow-up to assess additional rejections, patient survival, graft function and malignancy [28]. MMF recipients were less likely to experience another rejection episode, or graft loss or death, during the 3 years of study than were azathioprine group. The difference in the graft loss/death end-point value was accounted for by a lower incidence of graft loss in the MMF group, as patient deaths were equal between the group.

***Acute Refractory Rejection***

Acute refractory rejection is defined by the failure of repeated therapeutic attempts (usually with high dose corticosteroids and/or antilymphocyte agents) to reverse declining allograft function. MMF may be useful in treating refractory rejection because such rejection is thought to be mediated primarily by humoral immune responses.

Sollingen et al conducted a nonblind, noncomparative multicenter pilot study in 75 renal transplant patients to evaluate MMF for the treatment of acute refractory rejection. Patients with biopsy-proven rejection that was refractory to treatment with at least one course of antilymphocyte therapy, with or without prior treatment with high dose corticosteroids, were enrolled and treated with MMF 1 to 1.5 gm twice daily. Successful rescue therapy was defined as stabilisation or improvement in renal function, which was achieved in 69% of patients receiving MMF. Success was more likely in patients with better renal function at the initiation of mycophenolate mofetil treatment.

A subsequent 6-month randomised, nonblind trial compared MMF with high dose intravenous corticosteroids for treatment of acute refractory rejection in 150 first or second cadaveric renal transplant recipients; the primary efficacy end-point was graft and patient survival [29]. A total of 150 patients were enrolled and randomised in a 1:1 ratio to receive oral MMF 1.5 gm BD (n = 77) or intravenous methylprednisolone 5 mg/kg for 5 days (n = 73), tapered over the subsequent 5 days to 20 mg/day or the baseline dose of steroid given on the day before the diagnosis of rejection. Graft loss and death were reduced by 45% in the MMF treatment group (MMF: 14.3%; steroid: 26%; p: 0.081).

For the steroid group, 64.4% of patients had either subsequent biopsy proven rejection, presumptive rejection or treatment failure. This was statistically significant when compared with the MMF group (39.0%). At 12 months, a significant difference between groups was observed, with 31.5% of steroid group experiencing graft loss or death, compared with only 18.2% of the MMF group (p 0.042).

A meta-analysis comparing MMF with tacrolimus in refractory renal allograft rejection trial showed that the two drugs had similar effects on graft and patient survival and renal function [30]. Both drugs were superior in efficacy to corticosteroid treatment.

**(c) Treatment of Chronic Rejection**

Chronic rejection, or chronic allograft nephropathy, is defined as slow decline in renal function usually associated with intimal arterial thickening, interstitial fibrosis and proteinuria, and appears to have both immune and non-immune components. It is the most common cause of late graft loss. MMF seems to play a role in management of chronic rejection.

Weir MR et al performed a prospective clinical trial on treatment of chronic allograft nephropathy [31]. 28 cyclosporin A treated renal transplant

recipients with progressive deterioration of renal function were prospectively enrolled in the study and had their immunosuppressive regimen changed 24.3 ± 7.7 months after transplant. The cyclosporin A dose of all the patients was reduced by 50%. Azathioprine was discontinued and MMF (1 gm BD) was added to the regimen. The mean creatinine of the patients at the initiation of the change in immunosuppression was 3.5 ± 1.2 mg/dL (1.9 to 6.2 mg/dL). The observation period of follow-up after the change in immunosuppression was 7.2 ± 0.2 months.

Before the change in immunosuppression, the mean loss in renal function as indicated by the least-squares slope of the reciprocal of creatinine versus time was -0.006 ± 0.002 $(mg/dl)^{-1}$ per month. The change in immunosuppression significantly decreased the rate of loss in renal function for most patients when compared with their pretreatment values with a mean slope of 0.007 ± 0.003 $(mg/dl)^{-1}$ per month ($p = 0.003$). Renal function improved in 21 out of 28 patients. When cyclosporin A dose, mean arterial blood pressure, and baseline creatinine were adjusted by multivariate analysis, the change in immunosuppression was significantly associated with improved renal function ($p = 0.02$). There were no acute rejections after the immunosuppression change.

The same group described the longer follow-up of these patients [32]. The mean duration of follow-up was 15.6 ± 1.2 months. Twenty four of 32 patients had an improvement in the slope of decay of their renal function, whereas only 8 remained the same or decreased. Comparison of all pre-slopes versus post-slopes showed statistically significant improvement ($p = 0.005$). 10 patients had follow-up biopsies 1 year after immunosuppression change and eight of them had either stabilisation or improvement in the histological index of chronicity, whereas only 2 have progressed.

Fritsche L et al also described their experience of using MMF in treatment of chronic rejection [33]. 44 patients were enrolled in the study.

MMF was initiated 5.8 ± 4.8 years after renal transplantation. Before conversion to MMF, 32 patients were taking azathioprine. Concomitant medications were prednisolone and cyclosporin A/tacrolimus. The average decline of glomerular filtration rate (GFR) in the preceding 6 months before conversion was 2.3 ± 3.6 ml/min per month. The average loss of GFR was reduced significantly (0.27 ± 2.3 ml/min per month; p = 0.008). But there was no change in range of proteinuria after conversion to MMF.

All the above studies showed the benefit of addition of MMF with reduction of cyclosporin A dosage on chronic allograft nephropathy. If cyclosporin A dose was not reduced, the benefit of adding MMF to the immunosuppressive regimen was controversial [34-35].

At Princess Margaret Hospital, we tried to assess the effect of MMF on chronic allograft nephropathy of our Chinese renal transplant recipients [36].

**Methodology**

We retrospectively reviewed all cases of biopsy proven chronic allograft nephropathy for which MMF had been added to the cyclosporin A based immunosuppression. The rate of decline of renal function was indicated by the regression line (slope) of reciprocal of serum creatinine over time. The pre- and post- treatment slopes, cyclosporin A doses, cyclosporin A trough levels, blood pressure and cholesterol levels were compared.

**Results**

9 patients (4 patients had cadaveric renal transplantation; 5 patients had living-related donor renal transplantation) were given MMF for treatment of CAN from 1998 till July 2000 in our unit. The mean duration of MMF treatment was 36.0 ± 18.4 weeks. The mean dose of MMF at 6 months was 1.4 ± 0.2 g/day. The mean pre-treatment cyclosporin A dose and trough level were 161 ± 45 mg/day and 121 ± 18 ng/ml respectively. Reduction of post-treatment cyclosporin A dose and trough level (at 6 months) compared to

pre-treatment was 12.7% and 23.5% respectively ($p < 0.05$). The mean blood pressure and mean cholesterol level before and after treatment were also analysed (Table-6).

When post-treatment slope was compared to that of pre-treatment slope for each patient, 6 patients (67%) had improvement of the rate of decline of renal function. Among these 6 patients, 2 had less negative slopes while 4 had positive slopes. However 3 patients had more negative slope. The comparison of all post-treatment to pre-treatment slopes were summarised in Table-7.

**Conclusion**

This short term retrospective study showed that addition of MMF with reduction of cyclosporin A dose may have beneficial effect on the decline of renal function in our Chinese patients with CAN. Such findings were similar to those of Western studies. However the sample size of our study was not large. As a result, a long-term prospective study with larger sample size is required to confirm our preliminary results.

**(d) Use as a Cyclosporin-Sparing Agent**

Long term use of cyclosporin A carries the risk of renal function decline due to nephrotoxicity. The ability of mycophenolate mofetil therapy to allow cyclosporin dosage reduction and withdrawal has been evaluated in several studies in transplant patients with stable renal function and the results were promising.

In one nonblind, randomised controlled study, 159 patients were enrolled and MMF 2 g/day was introduced into their therapy regimens in addition to cyclosporin and corticosteroids, or replacing azathioprine in triple therapy [37]. For half of the patients, cyclosporin was withdrawn slowly over a period of 12 weeks until patients were cyclosporin- free and maintained on MMF and corticosteroids alone. The incidence of acute rejection episodes after

conversion to MMF was slightly higher in the cyclosporin-withdrawal group, but did not reach statistical significance. Renal function improved in the cyclosporin-withdrawal group. The creatinine clearance increased from 65.7 to 73.3 ml/min. For the control group, there was a slight decline of creatinine clearance from 65.6 to 61.4 ml/min.

There were also some studies comparing the effects of MMF with high or low dose cyclosporin [38-39]. The incidence of acute rejection episodes was not increased in the low dose cyclosporin group, suggesting that the addition of MMF to immunosuppressive regimen allows for a reduction in cyclosporin dosage.

However, there was one nonblind multi-centre trial reporting an increase in rejection rates in patients withdrawing cyclosporin while receiving MMF [40]. Patients enrolled in the study were receiving cyclosporin, MMF and prednisolone and they enjoyed stable renal function. They were randomised to withdraw cyclosporin (n = 30), withdrawal steroids (n = 34) or to remain on triple therapy (n = 30) 6 months after transplantation. Rejection in the following 6 months only occurred in the cyclosporin withdrawal group.

Based on the above studies, reduction of cyclosporin dosage may be possible in mycophenolate mofetil recipients. But further studies are required to resolve the issue of whether MMF can be used for long term maintenance of transplant recipients completely withdrawn from cyclosporin.

**(e) Use as a Corticosteroid-Sparing Agent**

Corticosteroids have numerous adverse effects, including new onset post-transplant diabetes mellitus, avascular necrosis, bone/joint complications, cataracts and post-transplant hypertension. All these adverse effects increase patient morbidity and mortality. Withdrawal or avoidance of corticosteroid after transplantation appear to have beneficial effects on blood pressure, lipid levels and quality of life [41-42].

Corticosteroid-sparing immunosuppression with MMF has been shown to have some success in small, mostly nonrandomised studies in nonsensitised patients [43-46].

There was one double-blind, randomised study comparing a standard corticosteroid dosage regimen (30 mg/day, tapered to 10 mg/day; n = 248) with a 50% reduced dosage (15 mg/day; n = 252) tapered to complete corticosteroid withdrawal [47]. In addition to steroid, MMF 2 g/day and cyclosporin were included in the immunosuppressive regimen. Despite the incidence of biopsy-proven acute rejection was higher in the corticosteroid-withdrawal group (23 and 26% at 6 and 12 months, respectively, vs 14 and 15% in the standard corticosteroid group, $p < 0.01$), the excess rejection episodes were mostly mild (Banff grade I) and reversible with corticosteroid therapy. The incidence of rejection episodes of Banff grade III or higher was similar in both groups. The overall incidence of rejection, graft loss and patient death in both groups was similar to that in the European renal transplant placebo-controlled study [15]. For those patient in the corticosteroid withdrawal group, they had significantly lower mean blood pressure and serum cholesterol and triglyceride levels than those in the standard dosage control group.

When induction therapy with antithymocyte globulin or muromonab CD3 in conjunction with MMF and cyclosporin, it appears to be beneficial in reducing the incidence of acute rejection in patients receiving low doses of corticosteroids. A subgroup analysis of the above randomised study showed that the incidence of biopsy-proven acute rejection among recipients of antilymphocyte induction therapy was similar in both the standard corticosteroid and corticosteroid withdrawal groups [48]. Birkeland SA, et al also described their results of retrospective study. In their study, corticosteroid use was avoided completely in renal transplant recipients receiving induction therapy with antilymphocyte globulin followed by maintenance therapy with MMF 2 g/day and cyclosporin 8 mg/kg/day

maintenance [49].

Chowdhury S et al devised a novel induction protocol combining the chimeric anti-IL2 receptor monoclonal antibody Bmab and MMF with immediate use of low-dose cyclosporin A and steroids [50]. The dose of MMF was 2 g/day and the dose of CsA was 2 mg/kg/day. CsA levels of 300 were reached only by day 10 while steroids were rapidly tapered to 5 mg/day by day 30. This protocol was compared to a standard induction therapy of OKT3 given for 7 to 14 days, MMF 2-3 g/day, high dose steroids and the introduction of full-dose CsA after a fall in creatinine.

The use of Bmab with MMF and early CsA therapy resulted in excellent patient tolerance and lower incidence of acute rejection (13% vs 39%, $p < 0.05$). The initial hospitalization period was shorter and there was fewer viral infections. In addition, the total steroid dose could be reduced by more than 40%.

However, there was one multicenter double-blind trial revealing that dual therapy of MMF and cyclosporin did not have equivalent efficacy to triple therapy with prednisolone in controlling acute rejection within the first year after renal transplantation [51]. The study aimed to enroll 500 patients, but discontinued enrollment at 226 patients since the incidence of rejection was higher in the corticosteroid withdrawal group than in the maintenance group (19 vs 5% at 1 year). The study did not report the use for antilymphocyte induction therapy and thus this may contribute to the lower efficacy of the maintenance immunosuppression after steroid withdrawal.

In conclusion, corticosteroid dose reductions appear to be possible with MMF, but complete withdrawal may be dependent on the previous use of antilymphocyte induction therapy to reduce the risk of rejection.

### (f) Use in High Risk Patients

***African-American patients***

African-American patients is a high risk group for renal allograft survival. This may be due to lower bioavailability of cyclosporin, differences in metabolism of corticosteroids and azathioprine, and poorer HLA matching with donors.

A subgroup analysis of the US mycophenolate mofetil renal transplant trial [16] revealed risk factor among Africa-American patient, especially in terms of poor donor-recipient HLA matching. There was no significant difference in the incidence of biopsy-proven rejection or therapy failure at 6 months post-transplant between the azathioprine group and MMF 2 g/day group. However, treatment of African-American patients with MMF 3 g/day resulted in a lower incidence of biopsy-proven rejection and treatment failure than did treatment with azathioprine. Despite treatment with MMF 3 g/day was associated with a higher frequency of adverse effects in the overall population, the better therapeutic efficacy of this dose in African-American patients may warrant its use.

***Patient with Delayed Graft Function***

Delayed graft function of transplantation is a major risk factor for long term graft survival. Efficacy of MMF in patients with delayed graft function was being determined by a subgroup analysis [52]. Rejection or treatment failure occurred in 37, 57 and 72% of patient with delayed graft function receiving MMF 2 and 3 g/day, and azathioprine/placebo, respectively. MMF 2 g/day was as effective in these patients as in patient with immediate diuresis. One year graft survival in patient with delayed graft function was better with MMF 2 g/day than with MMF 3 g/day or azathioprine/placebo group.

Three-year graft survival data were looked from the US and Tricontinental studies [16-17] comparing the 2 MMF dosages with azathioprine [52]. For those patients with delayed graft function, MMF 2

g/day produced a 76% graft survival rate at 3 years, as compared with 64% for MMF 3 g/day and 56% for azathioprine. In addition, for those patients with delayed graft function and biopsy-proven rejection, MMF 2 g/day significantly improved 3- year graft survival relative to azathioprine (75% vs 31%, p = 0.0092). Thus MMF (especially the 2 g/day dosage) has benefit in improving the outcome of those patients with delayed graft function, particularly in the context for acute rejection.

## Tolerability

Available data from 3 major randomised, double-blind, multicenter trials of primary use of MMF [15-17] suggest that gastrointestinal toxicity, certain types of haematological toxicity (especially leucocytopenia) and an increased incidence of some types of infection are the principal adverse effects of MMF. Rates of treatment discontinuation because of adverse events were higher with MMF 3 g/day and , to a lesser extent, MMF 2 g/day than with placebo [15]. However there were no difference between MMF and azathioprine (13% for MMF 2 g/day and 15% for MMF 3 g/day versus 14-16% for azathioprine) [17]. MMF 2 g/day was better tolerated overall than 3 g/day in renal transplant patients.

The tolerability profile for intravenous MMF is largely similar to that for the oral formulation. Phlebitis and thrombosis occur in about 4% of patients treated with intravenous MMF.

### *(i) Gastrointestinal toxicity*

MMF has been reported to cause more gastrointestinal adverse effects than azathioprine or placebo. Events that tended to occur more frequently with MMF included diarrhoea (with the higher dose), nausea, vomiting and gastroenteritis. Most gastrointestinal symptoms could resolve when the dosage was reduced.

The incidence of patients experiencing a gastrointestinal adverse event was greater in mycophenolate mofetil than placebo recipients in the major placebo-controlled trial (45.5% with MMF 2 g/day, 52.5% with MMF 3 g/day and 41.6% with placebo) [15]. When compared with azathioprine, MMF has been reported to have more gastrointestinal adverse effects [16-17].

Despite patients with active peptic ulcer disease and other serious gastrointestinal illnesses were excluded from the major MMF studies, gastrointestinal tract ulceration, haemorrhage or perforation occurred in a small number of patients treated with MMF (< 1 to 5%). Concomitant drugs

and/or infections may have contributed to these complications in some cases.

At Princess Margaret Hospital, the incidence of gastrointestinal problem was 10% for MMF group vs 5% for azathioprine group (p = NS) [26].

***(ii) Haemotoxicity***

Haematological adverse events, especially leucopenia and anaemia, occurred more in MMF than placebo recipients [15]. The percentage of patients experiencing ≥ 1 such event was 25.5, 23.8 and 13.3 in the MMF 2 g/day, MMF 3 g/day and placebo groups, respectively.

Leucopenia developed in 19% of patients receiving MMF 2 g/day, 30% of patients receiving 3 g/day and 30% of patients receiving azathioprine. The incidence of leucopenia relative to that in azathioprine recipients may depend on dosage.

Severe neutropenia (absolute neutrophil count < 500 /μl) developed in up to 2% of renal transplant recipients. It usually occurred at 1 to 6 months after transplantation.

At Princess Margaret Hospital, the incidence of leucopenia was 15% for MMF group vs 10% for azathioprine group (p = NS) [26].

***(iii) Infections***

All immunosuppressive therapies make the patients prone to infection. The incidence of opportunistic infection in those patients treated with MMF was higher than in those treated with placebo; and similar to or higher than in those treated with azathioprine. Viral infections tended to occur more frequently with MMF 3 g/day than with placebo or azathioprine. Such viral infections included cytomegalovirus tissue-invasive diseases, herpes zoster and herpes simplex infections. Fatal infection or sepsis occurred in less than 2% of patients treated with MMF 2 or 3 g/day.

At Princess Margaret Hospital, we have reviewed and analysed the incidence of CMV infection in our renal transplant recipients taking MMF (different dosages) or azathioprine. The incidence of cytomegalovirus infection was 14.6% in MMF group when compared to 10.7% in azathioprine group (p = NS). However, the incidence of cytomegalovirus infection was 27.8% in MMF 1 gm BD when compared to 7.1% in MMF 0.75 gm BD and 0% in MMF 0.5 gm BD (p = 0.04) [26].

***(iv) Malignancies and Lymphoid Disorders***

The risk of lymphoma and other malignancies, in particular those affecting the skin, is increased in patients receiving immunosuppressive therapy. The risk seems to be related to the intensity and duration of immunosuppression rather than the use of any specific agent.

For those patients who also received cyclosporin and corticosteroids, the incidence of malignancy did not appear to be significantly higher in patients treated with MMF than in those treated with placebo.

The overall incidence of lymphoproliferative disease or lymphoma was approximately 1% in MMF recipients.

***(v) Other events***

Metabolic disorders, like hyperkalemia and hyperglycemia, tended to occur less frequently with MMF than with azathioprine.

Haemoptysis occurred significantly more frequently with MMF 3 g/day than azathioprine, but was described as mild.

Allergic reactions to MMF have been reported rarely. Nephrotoxicity has not been reported with MMF.

***(vi) Teratogenecity***

Despite there are no current good controlled data from pregnant women,

teratogenic effects have been seen in animals with MMF dose ≤ 0.3 times lower than the clinical dose recommended for renal transplantation.

There was a case series of 3 women who received MMF during the early stages of pregnancy. Two of them gave birth to live infants without structural malformations and the remaining one had a spontaneous abortion. However the role of MMF in the latter event cannot be determined from present data [53].

## Conclusion

The goal of immunosuppressive therapy in renal transplantation is to maintain graft function by preventing graft rejection and its associated morbidities. Acute rejection still occurs despite the introduction of new immunosuppressive drugs and it remains as a serious risk factor for the development of chronic rejection ultimately leading to graft loss. The 1-year graft survival is no longer a sufficiently sensitive measure of efficacy of a new immunosuppressive agent due to the significant improvement in allograft survival in recent years. Thus the treatment and prevention of acute rejection should be measured as decreased rates for acute rejection will correlate with increased long-term graft survival. Mycophenolate mofetil seems to be a promising immunosuppressive agent.

Mycophenolate mofetil is an ester prodrug with higher bioavailability than the active agent, mycophenolic acid. Mycophenolic acid inhibits inosine monophosphate dehydrogenase, an enzyme that facilitates the conversion of inosine monophosphate to xanthosine monophosphate, a precursor of guanine nucleotides. This is an important step in the de novo pathway of purine nucleotide synthesis on which lymphocytes primarily depend.

As an antimetabolite immunosuppressant, mycophenolate mofetil has been evaluated for the prevention and treatment of acute rejection in renal transplantation. It is generally added to post-transplant therapy regimens in place of azathioprine, and in conjunction with cyclosporin and corticosteroids.

In large, randomised controlled trials in renal transplant recipients, mycophenolate mofetil has shown significant efficacy in reducing the incidence of acute rejection compared with azathioprine in the first year after transplantation. These studies have been carried out to 3 years post-transplant and there was a consistent trend towards improved outcomes over 3 years for patient treated with mycophenolate mofetil compared to the azathioprine or placebo group. Similar findings were found in our Chinese renal allograft recipients. Moreover, MMF was recently reported to reduce

late renal allograft loss at 4 years.

Mycophenolate mofetil has also been shown to reverse ongoing acute rejection episodes in renal transplant patients. It can be used to treat first acute rejection or acute refractory rejection.

In addition to acute rejection, mycophenolate mofetil may play a role in management of chronic allograft nephropathy. Addition of mycophenolate mofetil with reduction of cyclosporin A dosage will retard the progression of renal deterioration.

The efficacy of mycophenolate mofetil immunosuppression appears to allow sparing of other immunosuppressive agents, particularly cyclosporin and corticosteroids, in selected patients.

The main adverse effects are gastrointestinal tract events, haematological toxicity (especially leucocytopenia) and an increased incidence of some types of infections. Lower dosage (2 g/day) are generally better tolerated than higher dosages (3 g/day).

Pharmacokinetic and pharmacodynamic studies have demonstrated a good correlation between drug exposure (AUC) and the probability of rejection. As there is a more than 10 fold variability in pharmacokinetic profiles of mycophenolate mofetil, individualised MMF dose evaluation, guided by therapeutic drug monitoring, becomes the standard of practice at a growing number of transplant centers worldwide to optimise its efficacy.

Therapeutic drug monitoring of mycophenolate mofetil can be either pharmacokinetic or pharmacodynamic monitoring. Pharmacokinetic monitoring is direct measurement of plasma MPA concentration by either HPLC or EMIT immunoassay. EMIT immunoassay was found to have good precision and it is a simpler and not so labour-consuming when compared with HPLC. It has the further advantage of measuring the active metabolites M-2

as well and thus better reflect immunosuppression. Pharmacodynamic monitoring of biological effect of MMF provides an alternative to traditional therapeutic drug monitoring. This approach has significant advantages over the measurement of drug concentrations, especially in multiple drug therapy, where assessment of appropriate therapeutic range may be difficult. Measurement of IMPDH activity is a good indicator of immunosuppression of MMF.

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| | **1 week n = 7** | **1 month n = 9** | **3 months n = 8** | **1 year n = 5** |
|---|---|---|---|---|
| MMF dose | 1 gm bid | 1 gm bid | 1 gm bid | 0.75 gm bid |
| Trough ug/ml | 0.82 ± 0.27 | 1.17 ± 0.41 | 1.24 ± 0.69 | 1.87 ± 0.14*+ |
| Cmax ug/ml | 10.82 ± 8.21 | 14.73 ± 6.44 | 14.93 ± 10.26 | 18.39 ± 10.51 |
| Tmax hours | 1.48 ± 1.29 | 1.52 ± 1.10 | 2.04 ± 1.32 | 0.95 ± 0.40 |
| AUC ug/ml * hr | 26.14 ± 5.87 | 36.79 ± 5.75* | 43.19 ± 10.35* | 48.94 ± 18.21* |

* $p < 0.05$ vs 1 week
\+ $p < 0.05$ vs 1 month

Table-1 Pharmacokinetic parameters at different time periods

| Daily dose of MMF (mg) | Number of patients (N) | MPA level (μg/ml) | Coefficient of variance (%) |
|---|---|---|---|
| 500 | 3 | 0.57 ± 0.20 | 34.51 |
| 1000 | 7 | 1.67 ± 0.74 | 44.59 |
| 1500 | 7 | 5.71 ± 3.70 | 64.88 |
| 2000 | 66 | 4.21 ± 3.45 | 81.98 |
| 4000 | 4 | 3.71 ± 1.51 | 40.72 |

Table-2 MPA levels of different dosage groups

| | MPA level (μg/ml) | |
|---|---|---|
| **Time (min)** | **MMF dose 500 mg BD po** | **MMF dose 625 mg Q12H IV** |
| 0 | 0.14 | 2.21 |
| 15 | - | 4.92 |
| 30 | 0.31 | 10.10 |
| 45 | - | 9.75 |
| 60 | 9.74 | 12.69 |
| 90 | 4.95 | 10.61 |
| 120 | 4.30 | 8.77 |
| 180 | - | 4.54 |
| 240 | 3.13 | 1.85 |
| 720 | 0.14 | 2.21 |
| $AUC_{0\text{-}12hr}$ (μg·hr/ml) | 29.12 | 44.81 |

Table-3 Pharmacokinetic Profiles of MPA

| Time (min) | MPA level (μg/ml) | IMPDH activity (Cpm/min) |
|---|---|---|
| 0 | 0.14 | 2088 |
| 30 | 0.31 | 1442 |
| 60 | 9.74 | 1188 |
| 90 | 4.95 | 1918 |
| 120 | 4.30 | 2889 |
| 240 | 3.13 | 2449 |

Table-4 Correlation of MPA level and IMPDH activity for patient taking MMF 500 mg BD po

| Time (min) | MPA level (μg/ml) | IMPDH activity (Cpm/min) |
|---|---|---|
| 0 | 2.21 | 2700 |
| 15 | 4.92 | 1515 |
| 30 | 10.1 | 1512 |
| 45 | 9.75 | 1068 |
| 60 | 12.69 | 917 |
| 90 | 10.61 | 2584 |
| 120 | 8.77 | 2250 |
| 180 | 4.54 | 2667 |
| 240 | 1.85 | 2700 |

Table-5 Correlation of MPA level and IMPDH activity for patient taking MMF 650 mg Q12H IV

| | Pre-Rx | Post-Rx (at 6 months) | p value |
|---|---|---|---|
| CsA dose (mg/d) | 161 ± 45 | 143 ± 53 | < 0.05 |
| CsA trough level (ng/ml) | 121 ± 18 | 92 ± 26 | < 0.05 |
| MMF dose (g/d) | - | 1.4 ± 0.2 | - |
| Mean blood pressure (mmHg) | 142/82 | 134/80 | > 0.05 |
| Mean cholesterol level (mmol/L) | 6.64 | 6.36 | > 0.05 |

Table-6 Parameters before and after MMF conversion

| | Post-treatment | Pre-treatment | p value |
|---|---|---|---|
| $1 \times 10^{-3}$ ($\mu mol^{-1}yr^{-1}$) | $0.74 \pm 3.16$ | $-2.71 \pm 3.68$ | $< 0.05$ |

Table-7 Change of renal function before and after MMF conversion