The evolving role of mycophenolate mofetil in renal transplantation

A.N. WARRENS

From the Division of Medicine, Imperial College School of Medicine, London, UK

Pharmacology

Mycophenolate mofetil (MMF) is an ester prodrug with higher bioavailability than the active agent, mycophenolic acid (MPA). MPA inhibits inosine monophosphate (IMP) dehydrogenase, an enzyme that facilitates the conversion of IMP 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, unlike neutrophils, for example. In addition, different isoforms of IMP dehydrogenase exist, and MPA is not only almost five times more potent in inhibiting the type II isoform, that is associated with stimulated rather than resting lymphocytes, but is also, to some extent, cell type specific. Thus, on theoretical grounds, MMF represents a significant improvement on the antimetabolite azathioprine the use of which is well-established in organ transplantation.

In vitro and animal studies

MMF is effective in prolonging the survival of mouse and rat cardiac allografts, showing an additive effect with cyclosporin, brequinar or tacrolimus. It can also reverse rejection of rat cardiac allografts and prolong canine hepatic allograft survival as well as mouse and rat pancreatic allograft survival. Finally, in a canine model, it has been shown to prolong renal allograft survival and reverse acute rejection. Rat allograft studies have reported a role in reducing ischaemia-reperfusion injury. An additional experimental observation of potential interest in organ transplantation is the inhibitory effect of MPA on the proliferation of human arterial smooth muscle cells in vitro. Arterial disease plays a central role in chronic graft rejection, holding out hope for additional benefit from the use of MMF. In vivo MMF inhibits the graft coronary artery disease of rat cardiac allografts and cynomologous monkey-to-baboon cardiac xenografts and it attenuates functional, morphological and immunohistochemical changes associated with chronic rejection in rat renal allografts. Finally, in a rat model of progressive renal injury following 5/6 renal ablation, MMF was shown to limit a process that is not primarily immunological.

Also of interest is the observation that the use of MMF is associated with the induction of donor-specific tolerance in rat cardiac and mouse pancreatic islet is allografting.

The use of any immunosuppressive agent puts the patient at risk of increased infection and malignancy. With respect to infection, it is of interest to note that in a study in which rats were provoked with Pneumocystis carinii, no animal treated with MMF showed discernible infection, in contrast to rats treated with either tacrolimus or sirolimus, two other immunosuppressive agents used in transplantation. Interestingly, no case of P. carinii has been reported in any of the clinical trials of MMF in patients in the MMF limbs of the trial, although all three major trials have found P. carinii infection in the control (placebo or azathioprine) limbs. (Nor have any cases of Cryptococcus or Listeria infection been observed.)
reported in these trials.) This effect on *P. carinii* probably results from direct toxicity to the microbe by MMF.\textsuperscript{27}

Finally, MMF has also been demonstrated in animal models to have anti-neoplastic effects, such as the inhibition of the growth of human T-cell tumours in athymic nude mice.\textsuperscript{28}

**Clinical experience in renal transplantation**

**Prophylaxis**

All three of the large multicentre prospective, randomized, double-blind controlled trials of the use of MMF in clinical renal transplantation have demonstrated significant benefit from use of the drug. The European Mycophenolate Mofetil Cooperative Study Group\textsuperscript{24} reported a trial of the use of two doses of MMF (2 g/day or 3 g/day) vs. placebo in the prevention of acute rejection within the first 6 months following cadaveric renal transplantation. They recruited 491 patients, all of whom also received corticosteroids and cyclosporin. They found that the incidence of rejection, graft loss, death or other treatment failure in the first 6 months fell from 56% in the placebo group to 30% in the (better tolerated) 2 g/day MMF group. The MMF groups also used significantly less antilymphocyte therapy. The patients in the MMF groups also had lower serum creatinine levels at 6 months than those in the placebo group (although this might be an effect of survivor bias).

A second study (the ‘US trial’) compared the same two doses of MMF directly with azathioprine (1–2 mg/kg/day).\textsuperscript{26} The 499 patients recruited in this study also received corticosteroids, cyclosporin and antilymphocyte therapy at induction. This group reported that the incidence of rejection or treatment failure fell from 48% in the azathioprine group to 31% in each of the MMF groups. Of the rejection episodes, a higher percentage was classified as severe in the azathioprine group than in the MMF groups. Again, the MMF groups used much less antilymphocyte therapy.

The third study, conducted in Europe, Canada and Australia (the ‘Tricontinental Study’),\textsuperscript{25} also compared the same two doses of MMF directly with azathioprine (100–150 mg/day) in patients who were also receiving corticosteroids and cyclosporin in the first 6 months following transplantation. This trial recruited 503 patients. They found that the incidence of rejection or treatment failure fell from 50% in the azathioprine group to 38% and 35% in each of two MMF groups (2 g/day and 3 g/day, respectively). As in the US trial, the incidence of severe rejection (Banff grade II or higher) was less common in the MMF groups (6% and 10%) than in the azathioprine group (20%). Again, there was a lower use of antilymphocyte therapy in the MMF groups. A further evaluation at 12 months in this study showed that the significant difference between the MMF groups and the azathioprine group was maintained. As in the European trial, the patients in the MMF groups tended to have lower serum creatinine levels.

The data from the 1493 patients analysed in these three studies have been pooled and analysed together for outcome at 1 year.\textsuperscript{29} This analysis concludes that MMF can prevent about half of all acute rejection episodes. Graft loss due to rejection occurred in 6.3% of the placebo/azathioprine group and 2.6% and 3.5% of the MMF groups (2 g/day and 3 g/day, respectively). Graft loss from any cause was 12.4%, 9.6% and 10.8%, in the respective groups. Acute rejection (not necessarily resulting in graft loss) was 41%, 20% and 16% respectively. These findings all achieved statistical significance. In addition, a number of trends which did not achieve significance were noted: the severity of rejection was different in the three limbs, with the percentage with Banff grade II or III scores being 24%, 10% and 8% (placebo/azathioprine, MMF 2 g/day and MMF 3 g/day respectively); ALG was used in 20%, 9% and 5% of patients; and the MMF groups tended to have lower serum creatinine levels at 1 year.

The 3-year follow-up data have now been reported for these three trials, although there is, as yet, no analysis of pooled data. As summarized in Table 1, all studies show a decrease in graft loss from all causes, as well as graft loss due to rejection. The differences shown did not achieve statistical significance. However, none of the studies was designed or powered to detect differences in these parameters at 3 years, and it would, therefore, be incorrect to conclude that these constitute a meaningful negative result. Indeed, considering that all three reports show the same trends and the magnitude of the effect is consistent with what was reported at 1 year, the 3-year data are reasonably encouraging for the belief that MMF has a long-term benefit. It is not likely that a trial powered to detect differences of this magnitude at late time points will ever be undertaken.

There has been recent interest in the use of MMF with tacrolimus (FK506) at induction. However, anxiety has been expressed about the potential for toxicity with this régime. A recent report randomized 187 patients to receive either azathioprine or MMF with tacrolimus. All patients also received antilymphocyte therapy at induction and steroids. The incidence of acute rejection at 6 months in the MMF 2-g/day group was much lower (10%) than in the azathioprine group (33%), but there was no difference in toxicity.\textsuperscript{30} However, 6 months represents an
Table 1  The 3-year graft survival data for each of the arms of the three controlled trials of mycophenolate mofetil (MMF) in renal transplantation

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Azathioprine</th>
<th>MMF 2 g/day</th>
<th>MMF 3 g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Graft loss</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European study ⁵¹</td>
<td>22%</td>
<td>NA</td>
<td>15%</td>
<td>19%</td>
</tr>
<tr>
<td>American study ⁵⁰</td>
<td>NA</td>
<td>25%</td>
<td>19%</td>
<td>22%</td>
</tr>
<tr>
<td>Tricontinental study ⁵²</td>
<td>NA</td>
<td>20%</td>
<td>18%</td>
<td>15%</td>
</tr>
<tr>
<td><strong>Graft loss due to rejection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European study ⁵¹</td>
<td>11%</td>
<td>NA</td>
<td>5%</td>
<td>6%</td>
</tr>
<tr>
<td>American study ⁵⁰</td>
<td>NA</td>
<td>13%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Tricontinental study ⁵²</td>
<td>NA</td>
<td>10%</td>
<td>5%</td>
<td>3%</td>
</tr>
</tbody>
</table>

NA, not applicable.

extremely short follow-up period if there is an anxiety about neoplastic complications. Thus, although the early data are encouraging, the jury remains out on this question.

One final point may be made. Although the dose of 2 g/day showed a superior risk/benefit ratio for most patients, a higher dose may be more appropriate for Black patients. ⁵¹

**Rescue therapy**

MMF has also been used as ‘rescue therapy’ for acute rejection in uncontrolled studies. ³²,³³ In one multicentre, randomized, open-label study of 150 patients with biopsy-proven refractory rejection despite treatment with a biological agent (OKT3 or ALG), ³⁴ MMF was compared with intravenous corticosteroids plus azathioprine. All patients also received cyclosporin. The authors found that in the 6 months following enrollment, 26% of the steroids/azathioprine group suffered graft loss or death, compared with 14% in the MMF group. These figures were 32% and 18% at 12 months. There was also less need for subsequent antilymphocyte therapy in the MMF group.

A double-blind study has now been reported in which patients experiencing biopsy-proven rejection were randomized to receive MMF or azathioprine plus pulsed corticosteroids. ³⁵ At 6 months, there was a much higher requirement for subsequent antilymphocyte therapy in the azathioprine group (42%) than in the MMF group (17%). On the basis of these data, several centres have started to introduce MMF at first rejection.

**Maintenance therapy**

Another potential benefit of MMF may be that it allows the modification of the maintenance regimen, by reducing the toxic effects of other agents. The toxic effects of a large cumulative dose of corticosteroids is well recognized. A pilot study has reported the withdrawal of steroids from 26 patients between 4 and 30 months following transplantation and their subsequent maintenance solely on cyclosporin and MMF. ³⁶ This group observed no episode of acute rejection during a steroid-free follow-up which ranged from 7 to 18 months (mean 10 months).

As well as the anxieties associated with long-term steroid therapy, there are concerns that too many kidneys are lost as a result of chronic cyclosporin nephrotoxicity. One group described six patients with cyclosporin nephrotoxicity in whom it proved possible to stop cyclosporin and change the azathioprine to MMF without suffering any rejection episodes. ³⁷ Importantly, these patients showed a highly significant fall in their serum creatinine.

Even without frank nephrotoxicity, it has been shown that it is safe to reduce cyclosporin dosage following the conversion from azathioprine to MMF. Burke et al. reduced cyclosporin dosage to a target trough level of 50 ng/ml in 38 patients with impaired renal function, and noted a significant improvement in serum creatinine at 6 months. ³⁸ Weir et al. studied 28 patients with declining renal function. ³⁹ Their dose of cyclosporin was halved and MMF was substituted for azathioprine. Renal function improved in 21 of these patients (75%). There were no episodes of acute rejection in this study.

One report, compatible with the animal models of chronic rejection discussed above, observed that decreasing cyclosporin dose in the context of the introduction of MMF was associated with a reduction in the levels of the important fibrogenic cytokine TGFβ. ⁴⁰ However, a small retrospective case-control study failed to demonstrate any benefit of adding MMF to maintenance immunosuppression to patients with established chronic rejection. ⁴¹

One recent report describes the safety of conversion to MMF monotherapy in 13 patients with an improvement in serum creatinine. ⁴² However, this study reports only a very short follow-up period.
In summary, accumulating data suggest that MMF may have a role in the maintenance phase of the management of renal transplants.

Significance of the effect of MMF on the incidence of acute rejection

The previously preferred end-points of trials of the efficacy of maintenance immunosuppressive drugs, such as MMF, namely patient and graft survival, are now regarded as being less useful in assessing these agents: survival rates are so high on conventional treatment (with 80–90% 1-year graft survival) that it is difficult to perform a trial of sufficient power to detect differences of the magnitude expected in interventions of this nature. For this reason, surrogates for graft survival have been sought, and acute rejection is one commonly used index.29

Rejection is the principal cause of graft loss and early acute rejection has been recognized as ‘by far the most powerful predictor of graft loss’,41 including those losses due to chronic rejection. A study of 110 patients reported 1-year graft survival in three groups of patients—those without rejection, those with steroid-sensitive rejection, and those with steroid-resistant rejection—of 96%, 88% and 58% respectively.44 A review of 2322 transplant recipients demonstrated that the 1-year survival of those who suffer early acute rejection was 67%, compared with 86% for those who had no rejection.45 In an analysis of 603 patients, Naimark and Cole reported a 5-year graft survival of 67% in those who had experienced an early episode of graft rejection, compared with 86% in those who had not.46 A study of 219 patients considered the data in a different way, reporting that chronic rejection occurred in only 1/130 patients who had not experienced acute rejection, and 25/89 who had.47 Another report suggested that only 2.5% of patients who had been free of acute rejection eventually developed chronic rejection, compared with 12.5% of those who had suffered a single episode of acute rejection and 40% in those who had more than one episode.43 This group reported a half-life for graft survival of 16.9 years of those kidneys which had been rejection-free, but only 3.9 years in the group which had experienced more than one rejection episode. Focusing on chronic loss, Matas et al. calculated graft half-lives on the basis of studying all grafts still functioning at 1 year.48 They reported a half-life of 46 years in patients who had suffered no acute rejection, 25 years in those who had suffered one episode and 5 years in those who had suffered more than one.

Recent data have suggested that it is not merely the number, but also the severity of an acute rejection episode which affects the prognosis.49 In grafts which were functioning at 1-year post-transplantation, there was no difference in 8-year survival (89%) between those kidneys which had suffered no acute rejection and those which had had a mild rejection episode which had not been associated with loss of graft function. By contrast, those kidneys whose function remained impaired following a rejection episode had a much lower 8-year survival rate (69%).

Acute rejection is clearly not the whole story and better early surrogates for long-term prognosis are being developed. However, for all the reasons outlined above, an agent which decreases the frequency and severity of rejection episodes, such as MMF, seems likely to improve the lifespan of a renal allograft.

Adverse effects

The principal adverse effects of MMF reported in the transplantation studies are gastrointestinal (especially diarrhoea and vomiting), haematological and infective. In the European study,44 there was a small increase in each of these three categories of adverse effects compared with those patients on placebo. The marginal benefits on the incidence of rejection of using the higher dose (3 g/day) of MMF over the lower dose (2 g/day) were outweighed by increased adverse effects. The incidence of all gastrointestinal, haematological and opportunistic infective complications in the MMF 2-g/day group were 46%, 26% and 38% respectively, compared with 42%, 13% and 28% in the placebo group.

In the two studies in which MMF was compared with azathioprine, there was less variation in adverse effects between the MMF and control (azathioprine) groups. The US Study26 reported that opportunistic infections occurred in 46% patients in the azathioprine group and 45% and 47% patients in the two MMF groups. There was, however, more tissue-invasive CMV infection in the two MMF groups, in a dose-related pattern. Gastrointestinal adverse effects were more common in the MMF groups, but very rarely led to discontinuation of the drug. Three patients developed lymphoma or lymphoproliferative disease compared with none in the azathioprine group. Neutropenia was more common in the MMF group (4–5% vs. 2% on azathioprine).

In the Tricontinental Study,25 leucopenia and gastrointestinal disorders were more frequent in the MMF groups, and thrombocytopenia, thrombophlebitis and metabolic disorders were more frequent in the azathioprine group. However, infections occurred at similar frequencies in the three groups. Importantly, there was no difference in the incidence of tissue-invasive CMV infection between the MMF 2-g/day group and the azathioprine group.
overall incidence of non-cutaneous malignancies was 3.7% and 1.8% in the MMF 3-g/day and 2-g/day groups and 2.5% in the azathioprine group. Using drug withdrawal as an index of tolerability, MMF fared no worse than azathioprine, with 28% withdrawal in each MMF group, and 33% in the azathioprine group at 1 year.

The 3-year follow-up reports on toxicity from these three studies shows no additional toxic effects and are therefore also encouraging in this respect.

The incidence of severe adverse effects in the study of rescue therapy was higher in the MMF group than in the steroids/azathioprine group (56% and 31% respectively). This included a higher incidence of tissue-invasive CMV.

Conclusions

On the basis of the three large studies described above, it is clear that MMF does indeed decrease the frequency and severity of rejection. It undoubtedly improves graft survival at 12 months and the data at 3 years suggest that the benefits are maintained. It can be used as rescue therapy or merely as a prophylactic against future events in those who have already experienced rejection. It may also have a role in the prevention of chronic allograft nephropathy.

Although early data on its toxicity are encouraging, the suspicion that there is no such thing as a free immunological lunch makes one anxious that there may yet be a price to pay for this increased efficacy. It is therefore essential to stay vigilant, while remaining optimistic.

References


