The influence of mycophenolate mofetil on the incidence and severity of primary cytomegalovirus infections and disease after renal transplantation

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Abstract

Background. Mycophenolate mofetil (MMF) is increasingly used for immunosuppression after renal transplantation (RTx). The aim of our study was to investigate if the use of MMF has resulted in an increase in the frequency and severity of primary cytomegalovirus (CMV) infections.

Methods. Retrospective study of adult RTx patients who were CMV seronegative and who received a kidney of a CMV seropositive donor in the period 1992–1997 (n = 84). Twenty-four of these patients were treated with MMF (in combination with cyclosporin and prednisone; MMF+) and the other 60 were the control group (cyclosporin and prednisone; MMF−). No CMV prophylaxis was given. CMV infection was defined as CMV seroconversion of IgG antibodies. CMV disease was defined as CMV infection and fever in combination with one or more of the following: leukocytopenia, thrombocytopenia, elevated alanine aminotransferase, or histological evidence of tissue invasive disease.

Results. The incidence of primary CMV infections was similar in both groups (MMF+, 75%; MMF−, 63%). CMV disease was more frequent in the MMF+ group than in the MMF− group (67 vs 30%, P < 0.05). In the patients with CMV disease, the use of MMF did not affect severity of symptoms, frequency of tissue invasive disease, or frequency or duration of treatment with ganciclovir.

Conclusions. Addition of MMF to the immunosuppressive therapy after RTx did not result in an increase of primary CMV infections. However, these CMV infections led more often to CMV disease in patients treated with MMF than in those without MMF.

Keywords: cytomegalovirus; kidney transplantation; mycophenolate mofetil; primary cytomegalovirus infection

Introduction

The immunosuppressive drug mycophenolate mofetil (MMF) is increasingly used in the field of organ transplantation. MMF has reduced the incidence of acute rejection by more than 50% in the first year after renal transplantation (RTx) [1–3]. Only a slight increase in herpes simplex virus infections but no significant increase in the incidence of CMV infection/disease and other opportunistic infections was reported in studies comparing MMF with placebo or azathioprine (AZA) in combination with cyclosporin (CsA) and prednisone [1–3]. There was a slight but not significant increase in tissue invasive disease in patients treated with MMF 3 g/day compared with placebo [1]. It was our impression, however, that concurrently with the introduction of MMF we encountered more cytomegalovirus (CMV)-related problems than before. The aim of our study was to investigate if the addition of MMF to CsA and prednisone has resulted in an increase in the frequency and the severity of primary CMV infections in a high-risk population.

Subjects and methods

We performed a retrospective study of adult RTx patients who were CMV seronegative before RTx and who received a kidney of a CMV seropositive donor in the period of 1992–1997 at our hospital. Patients who had lost their renal graft within 2 months of transplantation were excluded. The minimum period of follow up was at least 12 months. The immunosuppressive therapy was assessed immediately after the start of transplantation. Patients treated with the combination of CsA and prednisone or with MMF, CsA, and prednisone were included for analysis. A total of 84 patients were identified. Twenty-four of the patients were treated with MMF in combination with CsA (target trough levels 150–300 ng/ml) and prednisone (MMF+ group). Six of them participated in a randomized controlled trial and their daily dose of MMF varied between 0.9 and 4.2 g (median 2.6 g) [4]. These patients received a graft between August 1994 and March 1995. In the remaining 18 patients a transplantation was performed between January 1997 and
January 1998 and they were treated with a standard MMF dose of 2 g/day. The control group was treated with prednisone combined with CsA (MMF− group; n = 60) and they received a graft between January 1992 and January 1997. A change in basic immunosuppressive therapy was recorded when this change lasted at least 1 month. Anti-rejection treatment consisted of intravenous administration of 1000 mg methylprednisolone on 3 consecutive days. If this treatment failed, anti-T-lymphocyte therapy (antithymocyte globulin or monoclonal anti-T-cell antibodies) was instituted. No CMV prophylaxis was given in either group. CMV IgM/IgG antibodies and other laboratory parameters were assessed at time of transplantation and in case of clinical symptoms (i.e. fever, abdominal pain, etc.) of CMV infection. Patients were treated with ganciclovir intravenously for 14 days in case of fever persisting for more than 2 weeks, or when tissue invasive disease or signs of CMV pneumonitis were present. In case of a relapse of CMV disease a second course of ganciclovir was administered.

Definitions of CMV infection/disease

CMV infection was defined as seroconversion of CMV IgG antibodies. These antibodies were determined by a sensitive ELISA [5]. CMV disease was defined as CMV infection and fever for at least 2 days with one or more of the following: leukocytes < 3.0 × 10^9/l, thrombocytes < 100 × 10^9/l, alanine aminotransferase (ALAT) > 50 U/l (normal value: < 35 U/l) or histological evidence of tissue invasive disease. CMV tissue invasive disease was defined as the histological finding of intracytoplasmatic inclusion bodies surrounded by inflammatory cells.

Statistics

Comparison of numerical data between the MMF+ group and the control group was carried out with the Wilcoxon’s rank sum test. Proportions were compared with chi-square analysis. The analysis was done on an intention to treat basis unless otherwise stated. A P-value < 0.05 was considered statistically significant.

Results

Clinical characteristics

The mean age of the patients in the MMF+ group was 7 years higher than in the control group. Sex and number of retransplants were equally distributed between the two groups (Table 1). There was no difference in the use of antirejection treatment in the first 3 months after RTx between both groups, although anti T-cell therapy tended to be used less frequently in the MMF+ group (P = 0.16). In twelve patients the immunosuppressive regimen was changed during the first 3 months after RTx (MMF+, n = 4; MMF−, n = 8). The median value of the interval between RTx and this change in therapy was 39 days (range 14–63 days). Three patients in the MMF+ group discontinued MMF because of intolerance to this drug (bone-marrow suppression or gastrointestinal complaints). In one patient in the MMF+ group, the immunosuppressive therapy was reduced to the continuation of MMF and prednisone because of intolerance to CsA. In four patients in the control group CsA was converted to AZA because of CsA nephrotoxicity. Finally, in four patients in the control group MMF or AZA was added after a severe rejection (AZA, CsA and prednisone (n = 3), or MMF, CsA and prednisone (n = 1)). All these patients were included in the analysis.

CMV infection/disease

CMV seroconversion was detected in 18 of the 24 MMF-treated patients and in 38 of the 60 patients who received no MMF. In 28 patients the CMV IgG antibodies remained negative (MMF+, n = 5; MMF−, n = 11) or were not measured again after transplantation (MMF+, n = 1; MMF−, n = 11), because there was no clinical reason to do this. Thus, the observed incidence of primary CMV infections (assuming that CMV IgG antibodies remained negative in cases where it was not measured) was 75% in the MMF+ group and 63% in the MMF− group (Figure 1). The maximum incidence of CMV infection (assuming that CMV IgG was positive in the patients where it was not measured) can be calculated as 79% in the MMF+ group and 82% in the MMF− group. CMV disease, however, was more frequent in the MMF+ group than in the MMF− group (67 vs 30%, P < 0.05) (Figure 1). This means that in the MMF+ group 84–89% of the primary CMV infections were symptomatic (=CMV disease) compared with 37–47% of the primary CMV infections in the MMF− group. The severity of the symptoms, frequency of tissue-invasive disease and frequency or duration of treatment with ganciclovir in both groups of patients with CMV disease were similar (Table 2). The first manifestation of CMV disease appeared at the same interval after transplantation in both groups (MMF+, median 49 days (range 33–116) vs MMF, median 51 days (range 29–259)). In 90% of the patients with CMV disease the first symptoms appeared within 3 months after RTx.

Restricting the above analysis to patients who did not receive antirejection treatment (MMF+, n = 14; MMF−, n = 31), or to the patients who remained on the initial immunosuppressive regimen during the first 3 months after RTx (MMF+, n = 20; MMF−, n = 51) gave the same results (data not shown).

Discussion

In this retrospective study, the addition of MMF to CsA and prednisone did not appear to result in an increase of CMV infections in CMV seronegative patients who received a renal transplant of a CMV seropositive donor (D+/R− combination). However, these primary CMV infections were nearly twice as often symptomatic in patients treated with MMF compared with patients not treated with MMF. As a
Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>MMF + group</th>
<th>MMF − group</th>
<th>(P)-value</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>47 ± 13</td>
<td>40 ± 15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>67</td>
<td>62</td>
<td>NS</td>
</tr>
<tr>
<td>First transplant (%)</td>
<td>87</td>
<td>90</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-rejection treatment (&lt;3 months after RTx):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\geq 1) anti-rejection treatment (%)</td>
<td>33</td>
<td>35</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-T-cell treatment (%)</td>
<td>4</td>
<td>15</td>
<td>NS</td>
</tr>
</tbody>
</table>

Underestimation of the frequency of CMV disease on the other hand seems unlikely, because our patients were instructed to contact us in case of fever or other clinical symptoms. Taking these factors into consideration thus strengthens the conclusion that CMV infection more often resulted in CMV disease in patients treated with MMF.

The rate of antirejection treatment during the first 3 months after RTx did not differ between the groups. Our study design was not suited to detect differences in acute rejection incidence between both immunosuppressive treatment regimens, however, because patients with early graft loss were excluded from analysis. On the other hand, there was a tendency to increased use of anti-T-cell therapy for treatment of acute rejections in the MMF− group, which increased net immune suppression in this group. However, excluding all patients treated with anti-T-cell therapy from the analysis did not alter the conclusions.

In accordance with our data, a case control study reported no difference in the frequency of CMV infections between patients on MMF or AZA in combination with CsA and prednisone [6]. Unfortunately no data were given on the symptomatology of these infections and it is unclear if anti-viral prophylaxis was administered. Other studies reported only a slight increase of CMV disease and other herpes infections in patients on MMF [1–3]. The discrepancy with our findings can be explained by differences in the study populations. Our study was limited to CMV D+/R− combinations, which is considered a high-risk population for CMV infections. In addition, in contrast to common practice in many transplant centres, our patients did not receive anti-viral prophylaxis. These factors may have contributed to a higher frequency of CMV infections and disease in our patients.

It is unclear which factors determine whether CMV infections become symptomatic in immune compromised hosts. Both direct cytotoxicity of the virus due to a decreased immune surveillance of the host and immunopathogenicity of the immune response of the host seem to contribute to symptomatology. In our study MMF was added to basic immunosuppressive therapy with CsA and prednisone. The increase in symptomatic CMV infections in the MMF+ group might therefore be explained by an increase in net immune suppression. On the other hand, literature data and our experience indicate that the use of MMF is not accompanied by
an increase in other (i.e. bacterial or fungal) infections. This argues against general attenuation of the immune response being the sole factor responsible for the increased incidence of CMV disease, and suggests that addition of MMF induces a specific change in the primary immune response to CMV infections (and probably other herpes viruses as well), which leads more frequently to symptomatic CMV disease.

MMF has several effects on the cellular and humoral immune system that might explain a change in coping with primary CMV infections. Mycophenolic acid, the active metabolite of MMF, decreases lymphocyte proliferation to several mitogens in various in-vivo and in-vitro studies by inhibiting inosine monophosphate dehydrogenase (IMPDH), which results in depletion of intracellular GTP and deoxy GTP pools [7]. Lymphocytes are heavily dependent on IMPDH for guanosine synthesis, since they lack the salvage pathway [7]. The depletion of intracellular GTP also blocks the transfer of mannose and fucose to membrane glycoproteins, which impairs the adherence of lymphocytes to endothelial cells [8]. It is not known whether proliferation of natural killer cells is affected by MMF.

Recovery of primary CMV infection has been shown to depend on expansion of natural killer cells and activated viral specific cytotoxic T lymphocytes [9]. MMF may have a specific depressant effect on proliferation of natural killer cells or CMV-specific cytotoxic T cells. Diminished adherence of these cells due to a change in membrane glycoproteins of the host might also inhibit recruitment of these cells at the site of infection.

MMF also decreases the humoral immune response in in-vitro and in-vivo studies (7). RTx patients treated with MMF have a decreased antibody response to antihymocyte globulin and to vaccination with influenza virus haemagglutinin [10,11]. There is indirect proof that formation of CMV antibodies has a disease mitigating effect after CMV infection, since several studies showed a reduction in the incidence of CMV disease after prophylactic administration of CMV immune globulin [12,13]. Although our retrospective data do not allow definite conclusions on this subject, our experience suggests a delay in antibody formation in MMF treated patients with CMV disease. Of course, this may be explained as an epiphenomenon of a weaker cellular immune response.

In conclusion, addition of MMF to basic immunosuppression after RTx did not result in an increase in the incidence of primary CMV infections. However, these infections were nearly twice as often symptomatic than without MMF. The severity of these symptomatic CMV infections was similar to that in patients not treated with MMF. MMF may cause some specific change in the primary immune response to CMV infections. Further studies are warranted to elucidate this change.

References

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