Nephrol Dial Transplant (1999) 14: 2710–2713

Nephrology Dialysis Transplantation

Original Article

Effect of mycophenolate mofetil on erythropoiesis in stable renal transplant patients is correlated with mycophenolic acid trough levels

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Abstract

Background. Both mycophenolate mofetil (MMF) and azathioprine (AZA) are immunosuppressive drugs that inhibit purine synthesis. In theory, MMF selectively inhibits lymphocyte proliferation, while AZA has well-known effects on red blood cells and thrombocytes as well. In renal transplant recipients we replaced CsA therapy by MMF in an attempt to reduce the immunosuppressive load 1 year after kidney transplantation. During this study we observed the effect of MMF on haematological parameters such as haemoglobin (Hb), leukocytes, and thrombocytes.

Methods. One year after kidney transplantation 26 stable patients were converted from cyclosporin A (CsA) to MMF (2 g/day). Thereafter, these patients were tapered twice in their MMF dose from 2 g to 1.5 g (4 months after conversion) and from 1.5 to 1 g (8 months after conversion) per day. The Hb levels, leukocyte and thrombocyte counts, and mycophenolic acid (MPA) trough levels were routinely measured.

Results. After conversion from CsA to MMF not only creatinine levels and the number of leukocytes, but also the haemoglobin (Hb) level significantly decreased in 21/26 patients ($P = 0.0004$). In eight patients the Hb level dropped more than 1 mmol/l (= 1.61 g/dl). Only in two of eight patients was an explanation for blood loss found. The effect on Hb level did not ameliorate after the first MMF dose reduction to 1.5 g/day. After tapering the MMF dose to 1 g/day, the Hb approached the pre-conversion level. Not only the MMF dose but also the mycophenolic acid (MPA) trough level correlated with the Hb level.

Conclusions. After conversion from CsA to MMF 1 year after kidney transplantation, a decrease in Hb level and leukocyte count was observed. The MPA trough level correlated also with the Hb level. The effect on the Hb level was reversible after dose reduction. This finding suggests that MMF exerts a negative effect on erythropoietic cells.

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Key words: cyclosporin A; haemoglobin; kidney transplantation; leukocytes; mycophenolate mofetil; mycophenolic acid

Introduction

Clinical trials in kidney transplant recipients have shown a higher efficacy of mycophenolate mofetil (MMF) and prednisone (Pred) compared to azathioprine (AZA) and Pred to prevent acute rejection [1–3]. Both MMF and AZA act on purine synthesis, aiming to decrease the proliferative response of allostimulated lymphocytes [4–7]. AZA and its metabolite 6-mercaptopurine (6-MP) act in a non-selective manner [4–6], and may cause pancytopenia. MMF, however, is a specific inhibitor of inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the purine synthesis of lymphocytes [7], and should theoretically have no or minimal effect on erythrocyte and thrombocyte proliferation.

During a conversion trial in renal transplant patients, we investigated the effect of conversion from cyclosporin A (CsA) to MMF on leukocytes, thrombocytes, haemoglobin (Hb), and serum creatinine. In addition we studied the correlation between mycophenolic acid (MPA) trough levels and the observed haematological side-effects. MPA is the active immunosuppressive metabolite of MMF and is formed following oral administration of MMF [8].

Subjects and methods

Patients transplanted between September 1995 and January 1997, with stable graft function and on maintenance treatment of CsA (±150 ng/ml CsA trough level) and Pred (10 mg/day) were converted to MMF (2 g/day) and Pred (10 mg/day) 1 year after kidney transplantation. In week 53 MMF treatment was started, while over a subsequent time-period of 4 weeks CsA was withdrawn. Four and 8 months after conversion, a reduction of 25% in MMF dose was performed in stable patients, reaching maintenance treatment of 1 g/day MMF and Pred.

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We report on 26 patients (12 male, 14 female; 6/14 females premenopausal) converted to MMF and reaching this low-dose maintenance immunosuppression without acute rejections, and observed the leukocyte and thrombocyte count in peripheral blood and the Hb level over time. In addition, fasting plasma MPA trough levels were measured (range 0–15 μg/ml, inter- and intra-assay coefficient of variation ⩽5%) (EMIT-Mycophenolic Acid Assay, Behring Diagnostics Inc. San Jose, CA, USA).

Statistical comparisons between different time-points were performed with the two-tailed Wilcoxon signed rank test. Correlation analyses were performed with the Spearman Rank test.

Results

After conversion from CsA to MMF (2 g/day) the median creatinine level of the total group decreased from 125 μmol/l (range 80–225) to 112 (68–247) ($P=0.0009$). Tapering the MMF dose to 1.0 g/day did not lead to a further change in the creatinine concentration, resulting in a median creatinine level of 113 (71–228) μmol/l at the end of the observation period.

After conversion from CsA to MMF the number of leukocytes decreased in 17/26 patients (median of total group: from 9.5 to 8.1×10^9/l) ($P=0.04$) (Figure 1**A**). These counts did not change after the MMF dose reductions to 1.5 g and 1 g. Consequently, the leukocyte counts measured during 1 g/day MMF were still lower in 21/26 patients compared to the counts during CsA therapy (median of total group: 8.5 *vs* 9.5×10^9/l; $P=0.01$). However, significant leukopenia (leukocytes $<4 \times 10^9$/l) did not develop.

After conversion from CsA to MMF and subsequent MMF dose reductions, the thrombocyte counts remained stable (median CsA, 211 (126–355); 2 g MMF, 222 (147–494); 1.5 g MMF, 222 (148–401); 1 g MMF, 222×10^9/l (128–295)). Thrombocytopenia (thrombocytes $<120 \times 10^9$/l) never occurred after conversion to MMF.

A significant decrease in the Hb level was found in 21/26 patients after conversion from CsA to MMF (median of total group, from 8.3 to 7.9 mmol/l (=from 13.4 to 12.7 g/dl); $P=0.0004$) (Figure 1**B**). Seven of 26 (27%) of the patients developed anaemia (male Hb <8.5 mmol/l (=13.7 g/dl); female Hb <7.5 mmol/l (=12.1 g/dl)). In five of these patients the drop in Hb was more than 1 mmol/l (=1.61 g/dl) and in three other patients this effect was also observed. Accordingly, in eight of 26 patients (31%) the decrease in Hb was more than 1 mmol/l (=1.61 g/dl). Only in two cases was an explanation for the drop in Hb level found. In both cases the anaemia was caused by menometrorrhagia, which was treated with iron supplementation and hormonal therapy. Haemolysis and significant faecal blood loss did not occur, and none of these patients needed a blood transfusion. The first dose reduction of MMF to 1.5 g did not positively influence the Hb level ($P=0.12$). After the second dose reduction to 1 g MMF, the Hb level in 20/26 patients was higher than the levels determined during 2 g MMF treatment, consequently the Hb levels reached the levels of pre-conversion ($P=0.75$).

We wondered whether not only the dose of MMF, but also the MPA trough levels were correlated with these side-effects of MMF. The MPA level was not correlated with the leukocyte counts (Spearman $r=-0.13$, $P=0.27$) (Figure 2**A**), while the MPA level was negatively correlated with the Hb level ($r=-0.35$, $P=0.002$) (Figure 2**B**). In addition, we found a signi-

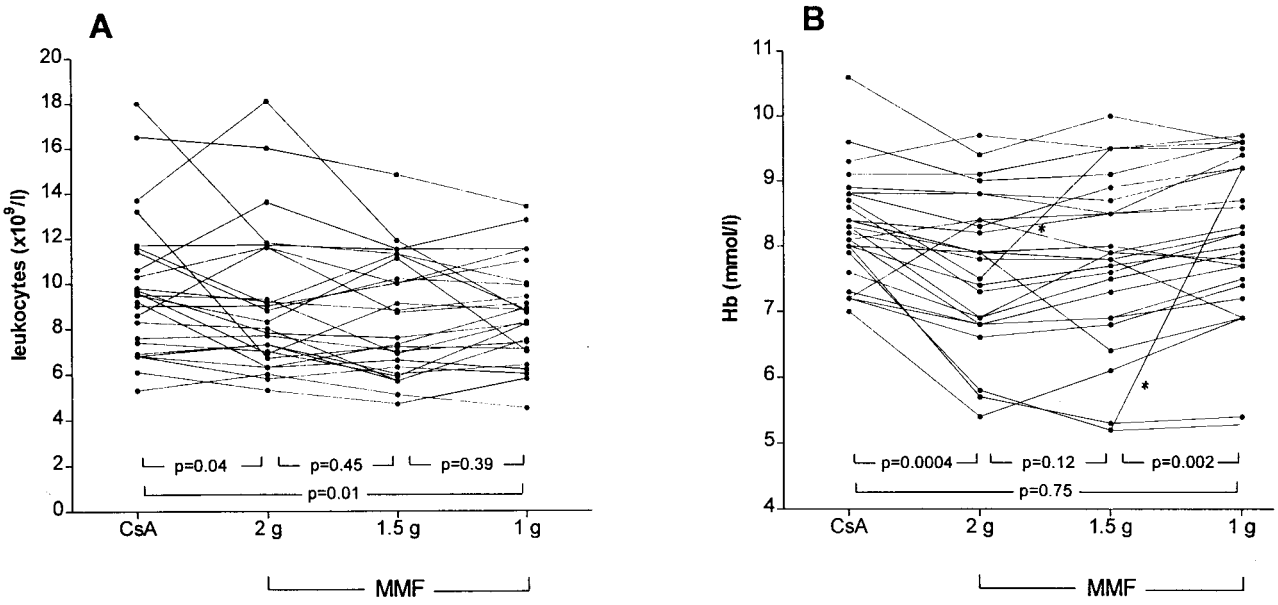

Fig. 1. (**A**) Leukocyte counts and (**B**) level of haemoglobin (Hb) before and after conversion from CsA to MMF therapy ($n=26$) and after dose-reduction of MMF. Conversion to MMF treatment was performed 12 months after kidney transplantation. Subsequent MMF dose reductions occurred 4 and 8 months thereafter (16 and 20 months post-transplantation). Shown are laboratory results of leukocyte counts from blood drawn 4 months after the change in treatment. *Menometrorrhagia.

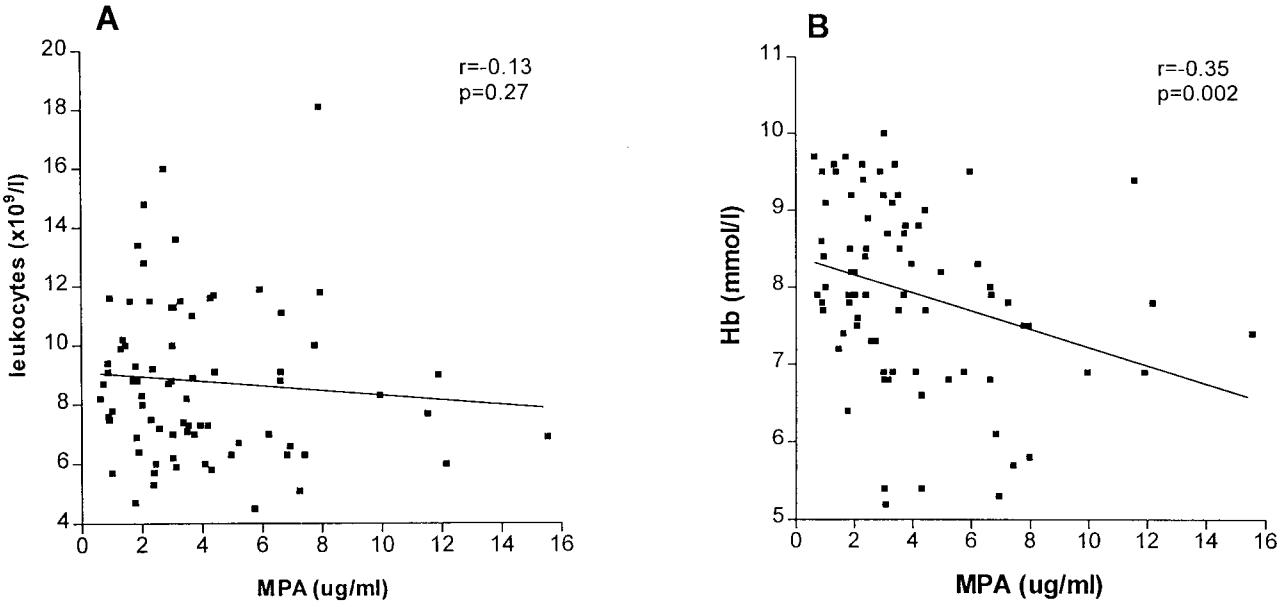

Fig. 2. Correlation between MPA trough level and (**A**) leukocyte count and (**B**) Hb level ($n=77$).

ficant negative correlation between the decrease of the Hb level from before to after conversion from CsA to MMF and MPA level after conversion (Spearman $r=-0.66$, $P=0.0003$) (Figure 3).

Discussion

The occurrence of haematological side-effects described using MMF in renal transplant patients seems to be dose dependent [3,9]. The 'European Mycophenolate Mofetil Cooperative Study Group' reported that leukopenia occurred in 13.8% of the patients receiving 3 g/day MMF and in 10.9% receiving 2 g/day MMF [9]. According to the 'Tricontinental Mycophenolate Mofetil Renal Transplant Study Group' 35% of the patients treated with 3 g/day MMF and 19% of the patients treated with 2 g/day MMF developed leukopenia [3]. The opposite holds true for the development of thrombocytopenia: with thrombocytopenia in 3.1% (European) and 5% (Tricontinental) of the patients treated with 3 g MMF compared to 4.2% (European) and 9% (Tricontinental) of the patients treated with 2 g MMF/day [3,9].

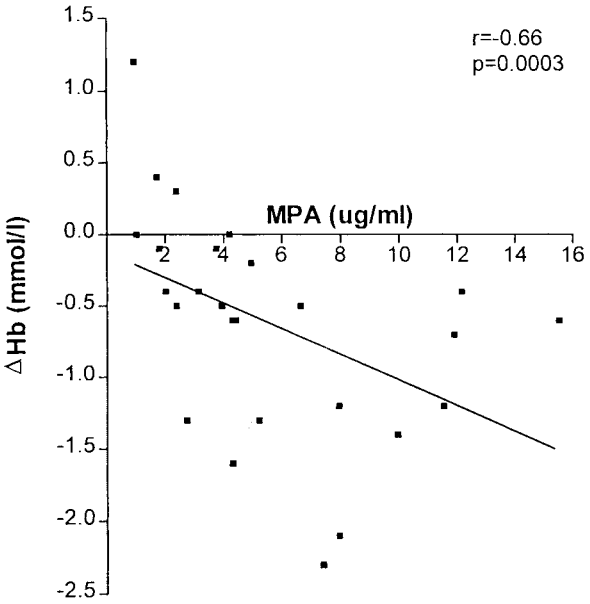

Fig. 3. Correlation between MPA trough level and the decrease of Hb level from before to after conversion from CsA to MMF ($n=26$).

However, it should be noted that these patients were treated with MMF, CsA, and Pred within the first year after kidney transplantation. The first months after transplantation are well known for the incidence of various infections e.g. cytomegalovirus (CMV) which may have caused a considerable number of episodes of thrombocytopenia and/or leukopenia. Our patients were converted from CsA to MMF 1 year after transplantation, and CMV disease or other infections are no longer a problem at this juncture. Additionally, apart from the conversion from immunosuppression other drug treatment was unchanged.

The 'European Mycophenolate Mofetil Cooperative Study Group' reported that 6.8% of the patients from the group treated with a daily dose of 3 g MMF developed anaemia. This percentage was 4.2% when patients were treated with 2 g MMF [9]. In the 'Tricontinental Mycophenolate Mofetil Renal Transplant Study Group' these percentages were 15 and 9% respectively [3]. However, no definition of anaemia was given.

In our series, in six of 26 patients (23%) the Hb level dropped with more than 1 mmol/l (=1.61 g/dl), while in 15 other patients the Hb level also decreased. Giving MMF in combination with CsA within the first days after transplantation may mask the effect of

MMF on erythropoietic cells, because of co-medication and infections compared to the situation of converting patients from CsA to MMF 1 year after transplantation. This is even more striking, as kidney function after withdrawal of CsA treatment improved with a median serum creatinine of 125 to 112 μmol/l and remained stable with a median creatinine of 113 μmol/l after tapering of MMF. Recently published data from our centre show, in a small number of patients, that those with adverse events have higher MPA trough levels than those without adverse events [10]. In the present study, we demonstrated that haematological side-effects such as the Hb level are also related to MPA trough levels. The importance of the MPA level was confirmed by the significant negative correlation between the decrease of the Hb level from before to after conversion from CsA to MMF and MPA level after conversion.

From these results we conclude that treatment with MMF does result in a decrease in Hb, that is reversible after dose reduction. This decrease in Hb was also correlated with MPA trough levels. In other words, we presume that MMF does not specifically inhibit lymphocytes alone, but also has an effect on erythropoietic cells.

Acknowledgements. This study was supported by grant C95.1472 from the Dutch Kidney Foundation.

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Received for publication: 18.2.99
Accepted in revised form: 7.6.99