CHAPTER 3

CONCENTRATION CONTROLLED TREATMENT OF LUPUS NEPHRITIS WITH MYCOPHENOLATE MOFETIL

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ABSTRACT

Background Mycophenolate mofetil (MMF) has recently been established as a potent drug in maintenance treatment for lupus nephritis. However, there is no consensus on the optimal dosing regimen due to a high inter-individual variability of mycophenolic acid (MPA), the active metabolite of MMF. This retrospective study aimed to investigate the effect of an individualized dosing regimen through concentration controlled treatment on MPA exposure and renal outcome in patients with lupus nephritis. Methods Sixteen patients with lupus nephritis and treatment with low dose intravenous cyclophosphamide followed by MMF were included. MPA area under the plasma concentration-time curve from 0 to 12 hours (MPA-AUC\textsubscript{0-12}) was assessed within a month after MMF initiation. After determination of MPA-AUC\textsubscript{0-12}, MMF doses were titrated to achieve a target MPA-AUC\textsubscript{0-12} of 60-90 mg*h/l. After on average six months, MPA-AUC\textsubscript{0-12} measures were repeated to assess the effect of dose adjustment. Results One month after introducing MMF, MPA-AUC\textsubscript{0-12} was low and showed a high inter-individual variability. Dose adjustment with a target MPA-AUC\textsubscript{0-12} of 60-90 mg*h/l resulted in individualized MMF dosing, significantly higher MPA-AUC\textsubscript{0-12} levels and a non-significant reduction in variability of MPA-AUC\textsubscript{0-12}. Adverse effects were reported by 37.5% of patients, which resulted in a switch to azathioprine in two patients. There was no significant relationship between the occurrence of adverse effects and MPA-AUC\textsubscript{0-12}. At 12 months of follow-up 87.5% of patients had achieved either partial (18.7%) or complete (68.8%) remission. Conclusion Concentration controlled dose adjustments with a target MPA-AUC\textsubscript{0-12} of 60-90 mg*h/l was associated with optimized MPA exposure and an excellent renal outcome at 12 months of follow-up in a small sample of SLE patients with lupus nephritis.
INTRODUCTION

Lupus nephritis is a prevalent organ involvement in systemic lupus erythematosus (SLE) and affects up to 60% of patients. Renal involvement is strongly related to a high morbidity and mortality in SLE, but early and intensive treatment can greatly improve renal outcome. For decades the first choice of treatment for severe lupus nephritis consisted of high doses intravenous cyclophosphamide (IVC) in combination with corticosteroids, known as the NIH regimen. This regimen with high IVC doses has shown variable results as a remission induction and maintenance therapy in proliferative lupus nephritis. In addition, the high incidence and severity of IVC related adverse effects has resulted in a search for less toxic alternative therapies. Among these alternatives, the Euro-Lupus regimen with low dose IVC as remission induction followed by azathioprine (AZA) as maintenance therapy has been shown to be an equally effective and safe therapy. Also at a 10 years follow-up, the Euro-Lupus regimen did not differ from the NIH regimen in terms of clinical outcomes.

Another frequently studied drug for treatment of lupus nephritis is mycophenolate mofetil (MMF). MMF as remission induction treatment has shown to be at least equivalent in terms of efficacy and safety compared to high dose IVC. In addition, some studies have reported better clinical outcome and less drug related adverse events with MMF. Although both MMF and AZA have been established as effective maintenance treatments, contradictory results have been published on the optimal maintenance regimen. One recent study found MMF to be superior to AZA in preventing renal flares in patients with a good response after 6 months induction treatment with either MMF or IVC. However, the MAINTAIN trail in which maintenance treatment with MMF was compared to AZA after induction treatment with low dose IVC showed no difference in the incidence of renal flares.

The inconsistent findings in the differences in clinical outcome between MMF and AZA maintenance therapy may be influenced by the fact that the optimal MMF dose in lupus nephritis is unknown and different dosing regimens have been applied. Although MMF has become an important drug in the management of SLE, it is not officially
registered for treatment of lupus nephritis and formal dosage recommendations are unavailable. As a result, dosages have been based on experience in the renal transplantation setting. In kidney transplantation patients, doses below 1 g/d have been associated with a higher risk of graft rejection\(^1\), while doses above 3 g/d have been related to an increased occurrence of drug related adverse effects.\(^2\) Therefore, MMF trials for lupus nephritis have applied dosages between 1 to 3 g/d and adjustments were made based on therapeutic response and side effects.\(^3\) In current clinical practice of maintenance therapy for lupus nephritis, MMF is generally administrated at a fixed starting dose of 2 g/d. However, studies in renal transplantation patients have also shown that the pharmacokinetics of mycophenolic acid (MPA), the active metabolite of MMF, exhibit a considerable variability between individuals and over time.\(^4\);\(^5\) A high inter-patient variability of MPA has also been found in patients with autoimmune diseases, including SLE\(^6\);\(^7\), and more specifically in SLE patients with lupus nephritis.\(^8\);\(^9\)

Because of these characteristics of MPA exposure and its associations with clinical outcomes, establishing individualized dosing regimens by means of therapeutic drug monitoring (TDM) is considered essential in MMF treatment in SLE patients.\(^10\);\(^11\);\(^12\)-\(^13\) In addition, therapeutic target levels of MPA area under the plasma concentration time curve (MPA-AUC) above 35 and 45 mg*h/l have been recommended to achieve good response based on retrospective data.\(^14\);\(^15\) To our knowledge, no study has reported on the actual implementation of such therapeutic target ranges and its influence on MPA exposure and treatment outcome. Therefore, the aim of the present study was to report our experience with optimized dosing of MMF with a target MPA-AUC\(_{0-12}\) of 60-90 mg*h/l after induction treatment with low dose IVC according to a modified version of the Euro-Lupus protocol in SLE patients with proliferative lupus nephritis.
METHODS

Patients

From 2005 onwards the patients presenting with proliferative lupus nephritis to the nephrology and rheumatology departments at the Leiden University Medical Centre (LUMC) were treated with low dose IVC (six pulses of 500 mg in three months) followed by MMF with a starting dose of 2 g per day. As part of local hospital policy, after determination of MPA-AUC\(_{0-12}\), MMF doses were titrated to achieve a target MPA-AUC\(_{0-12}\) of 60-90 mg*h/l. All included patients had SLE according to the revised American College of Rheumatology criteria.\(^{25}\) For this retrospective cohort study 16 patients were identified with a total of 28 registered MPA measurements. The majority of patients were of Caucasian descent (75%).

Pharmacokinetic analyses

MPA concentration measures were derived from blood samples that have been taken for therapeutic drug monitoring (TDM) purposes. Prior to sampling, patients had held a 12-hour overnight fast. Blood samples were taken before the administration of MMF morning dose and one, two, and three hours after intake.

Samples were analyzed for MPA by high performance liquid chromatography (HPLC). TDM was performed on the basis of the limited sampling strategy and Bayesian estimation of the MPA clearance using MW/Pharm version 3.5 (Mediware, Groningen, The Netherlands) as previously described.\(^{26}\) MPA oral clearance was used to calculate MPA-AUC\(_{0-12}\). Therapeutic dose adjustments based on MPA-AUC\(_{0-12}\) measurements were also recorded.

Outcome measures

The following disease activity parameters were recorded at the time of MPA exposure measurement: hemoglobin (Hb), serum and urinary creatinine levels, serum albumin levels, proteinuria, and glomerular filtration rate according to the Modification of Diet in Renal Disease (MDRD) study equations. In addition, serum creatinine, serum
albumin and proteinuria were registered three months prior to initiation of MMF treatment, and 0, three, six, nine, and 12 months after the start of treatment.

Treatment response was assessed at six and 12 months. The following three response categories were defined: 1) complete response: proteinuria below 0.5 g/day and stable serum creatinine levels or less than 25% higher than at the start of treatment, 2) partial response: more than 50% reduction in proteinuria and no increase in serum creatinine levels, and 3) failure: not reaching the criteria for partial response.

Statistical analyses

Data were analysed using SPSS software version 17. Descriptive statistics and frequencies were obtained for the patient characteristics. Independent t-tests were used to investigate differences in MPA exposure between first and second measurements and to assess changes in disease activity parameters. Associations between MPA-AUC_{0-12} and disease activity parameters were explored with Pearson correlation coefficients. ANOVA was used to test differences in MPA-AUC_{0-12} between treatment response groups. An alpha level of .05 was used for all statistical tests.

RESULTS

Patient characteristics

All 16 patients were treated with low dose IVC followed by MMF for an episode of proliferative lupus nephritis. Five patients were diagnosed with a class III, 11 with a class IV. This was the first episode of proliferative lupus nephritis for 10 patients and six patients experienced a renal flare. Previous episodes of lupus nephritis had been treated with IVC and corticosteroids (two), IVC and azathioprine (one), MMF and corticosteroids (one), or azathioprine and corticosteroids (two). 93.7% of patients used one or more anti-hypertensive drugs at time of treatment for lupus nephritis: 73.3% ACE inhibitors, 40.0% AT-II antagonists, 20.0% calcium antagonists, 20.0% diuretics, and 13.3% beta blockers.

Twelve patients had two or more measurements of MPA blood concentrations. The first measurement before dose adjustment was performed on average 32.6 (SD = 27.7) days after the start of MMF maintenance treatment. The second MPA levels that
were assessed after dose adjustment took place on average 6.6 (SD = 7.2) months after
the first measurement. Patient characteristics before dose adjustment are shown in Table 1.

| Male (N, %)    | 1 (6.3%) |
| Age in years (SD) | 33.2 (12.1) |
| Weight in kg (SD)  | 67.0 (11.5) |
| Serum albumin (SD) | 39.2 (5.7) |
| Serum creatinine (SD) | 98.6 (55.0) |
| Hemoglobin (SD)   | 7.1 (.99) |
| Proteinuria (SD)  | 1.3 (1.3) |
| MDRD (SD)         | 78.3 (36.8) |
| MMF dose g/day (SD) | 1.9 (.29) |

Before the start of MMF, four patients (25.0%) had already reached complete
remission, four patients (25.0%) showed partial remission and eight patients (50.0%) were
labeled as failures. After six months of MMF treatment, 10 patients (62.5%) had
completely responded, four patients (25.0%) showed a partial response, and two patients
(12.5%) were classified as non-responders. At 12 months, one patient had switched from a
partial to a complete response.

**Pharmacokinetics**

On the basis of the first MPA-AUC₀₋₁₂ measurement, dose adjustments were made
in 13 of 16 patients (81.3%). In two patients MMF dose was reduced and 11 patients
received a dose increase. MMF dose was on average 1.9 g (SD = .29) before and 2.6
(SD = .82) after first MPA-AUC₀₋₁₂ measurement. Figure 1 depicts the dose adjustments in
the 12 patients who had repeated MPA-AUC₀₋₁₂ determinations. The dose range was 1-2
g/24h before the first MPA-AUC₀₋₁₂ and 1.5-4 g/24h before the second MPA-AUC₀₋₁₂.
Figure 1. MMF dose/24h at first (1) and second (2) MPA-AUC0-12 (N = 12).

Figure 2a shows the mean MPA levels before and after dose adjustment for four different time points after MMF administration in the 12 patients who had at least two MPA-AUC0-12 measurements. Mean MPA level after dose adjustment was significantly higher one hour after MMF intake ($p = .023$).
The mean MPA-AUC\textsubscript{0-12} levels before and after dose adjustment are depicted in Figure 2b. Mean MPA-AUC\textsubscript{0-12} before dose adjustment was significantly lower than after dose adjustment (\(M = 46.5, SD = 24.3\) vs. \(M = 69.3, SD = 19.4; p = .018\)). In addition, MPA-AUC\textsubscript{0-12} levels tended to be less variable after dose adjustment (\(SD = 24.3\) versus \(SD = 19.4\)), although the difference in variances was not significant (\(p = .456\)).

MPA-AUC\textsubscript{0-12} was significantly correlated with levels at 0, one, two, and three hours after MMF administration (\(r = .79, .62, .60, .52\), all \(p < .001\)). There was no significant relationship between MPA-AUC\textsubscript{0-12} and serum albumin (\(r = .270, p = .212\)), proteinuria (\(r = -.18, p = .468\)), or creatinine clearance (\(r = -.275, p = .174\)).

Renal outcome

The efficacy of MMF therapy was evaluated by the follow-up of proteinuria, serum creatinine, and serum albumin levels. Twelve months after the start of MMF treatment proteinuria levels had significantly decreased (\(M = 2.18\) g/day, \(SD = 1.60\) vs. \(M = .72\) g/day, \(SD = .95; p = .007\)) (Figure 3). Serum creatinine remained stable over time (\(M = 92.38\) μmol/l, \(SD = 68.32\) vs. \(M = 92.00\) μmol/l, \(SD = 50.24; p = .986\)). Albumin levels showed a marked increase from a mean value of 38 g/l (\(SD = 5.31\)) to 43.0 g/l (\(SD = 3.82; p = .008\)).
Figure 3. Proteinuria (g/24h) at start of MMF treatment and at twelve months follow-up.

Adverse events

Adverse effects were reported by six patients (37.5%). One patient (16.7%) ended MMF treatment after three weeks because of ongoing nausea and vomiting and switched to AZA as maintenance therapy. Two patients (33.4%) also experienced gastrointestinal complaints such as nausea, cramps and diarrhea, but no dose adjustments were made. One patient (16.7%) switched to AZA after two years because of recurrent episodes of sinusitis. Recurrent infections were experienced by three other patients (50%) as well. Sleeping disturbances were reported by one patient (16.7%).

There was no significant relationship between the occurrence of adverse effects and MPA-AUC0-12 ($p = .293$).
DISCUSSION

This is the first study to report on the effect of MPA concentration controlled treatment on MPA exposure and renal outcome in a cohort of SLE patients with proliferative lupus nephritis. The findings indicate that adjusting MMF dose aimed at a target MPA-AUC0-12 of 60-90 mg*h/l results in individualized MMF dosing with increased MPA exposure and decreased inter-individual variability. In addition, this individualized dosing regimen of MMF in the context of a modified version of the Euro-Lupus protocol was associated with a good renal outcome with 87.5% of patients showing partial or complete response after 12 months of MMF treatment.

MMF has recently been established as an effective drug in both the induction and maintenance treatment of lupus nephritis. However, it remains unclear whether it is superior to alternative therapies such as high dose IVC or the Euro-Lupus regimen with low dose IVC followed by AZA. In the present study, patients were treated according to a modified version of the Euro-Lupus regimen with low dose IVC followed by MMF instead of AZA. A recently published long-term study of the ALMS group found MMF to be superior to AZA in maintaining renal response to treatment and in preventing renal relapse. In addition, fewer patients in the MMF group withdrew due to adverse effects. However, most previous studies failed to find differences in efficacy or adverse events between MMF and AZA maintenance therapy. Among these studies is the long-term MAINTAIN Nephritis Trial, which did find fewer renal flares in the MMF group (19% vs. 25%), but this difference was not significant.

Studies investigating the difference in clinical outcome between MMF and AZA maintenance therapy administrated MMF at a fixed dose. However, studies into the pharmacokinetics of MMF have suggested that results with MMF may be further improved through concentration controlled treatment. Because exposure to MPA, the active metabolite of MMF, has been found to have a high inter-individual variability, concentration controlled treatment is considered to have a pivotal role in MMF therapy. This high inter-individual variability has been reported across various patient groups.
including renal transplantation30, autoimmune disease in general18,22, and SLE19,24 and lupus nephritis in particular.20,22 Guidelines for therapeutic target ranges for MMF therapy in SLE patients have been proposed for MPA-AUC0-1221;31 and trough levels.19,22 MPA-AUC0-12 levels above 45 mg*h/l have been shown to precisely distinguish responders from non-responders in lupus nephritis patients who were treated with MMF and prednisone.21 In addition, a more precise differentiation of MPA-AUC0-12 levels was associated with response rates of 60 and 100% for MPA-AUC0-12 levels of 30-60 mg*h/l and > 60 mg*h/l, respectively.21

Although pharmacokinetic monitoring based on MPA-AUC0-12 levels is considered to be the golden standard to measure MPA exposure, the application in real life is impractical because of the numerous blood samplings. Limited sampling strategies up to three hours after MMF administration32 and even single point trough levels have been shown to be good alternatives in patients with SLE.19,22 The present study used sampling times up to three hours after MMF intake to calculate MPA-AUC0-12 and showed that concentration controlled treatment with a target MPA-AUC0-12 of 60-90 mg*h/l resulted in exposure within the target range. Although MPA-AUC0-12 levels were low with a mean of 46.5 mg*h/l before dose adjustment, MPA-AUC0-12 levels increased to an average of 69.3 mg*h/l after dose adjustment. In addition, inter-individual variability in MPA exposure tended to be lower on second measurement of MPA-AUC0-12 levels. But also levels at 0, one, two, and three hours after MMF administration showed significant associations with MPA-AUC0-12 levels. Hence, both limited sampling strategies in combination with population pharmacokinetics as well as single point trough levels are potential alternatives to the extensive MPA-AUC0-12 measurements. The choice for one method over the other could be based on the availability of resources and/or personal preference of the patient or treating physician.

Another alternative for TDM that has not been addressed in the present study, is the use of inosine 5’monophosphate dehydrogenase (IMPDH). IMPDH is a rate-limiting enzyme and inhibition by MPA results in a decreased proliferation and recruitment of monocytes and lymphocytes to areas of inflammation.33 IMPDH has been suggested as a
promising biomarker of MPA pharmacodynamic activity in renal transplant patients and childhood-onset SLE patients, with an additional role in determining MMF starting dose in the SLE group. However, studies with less specific cohorts of SLE patients have not been performed and no standardized analytical protocol exists to determine IMPDH. Hence, more studies are needed to validate the use of IMPDH in TDM in SLE patients with lupus nephritis.

Previous studies have indicated that the variability in MPA exposure between SLE patients cannot be explained by differences in MMF dose. Instead, associations have been found for creatinine clearance, albumin levels, and immunological markers (i.e., anti-dsDNA and complement). Although comparable determinants of variability have been reported in renal transplantation patients, the most important influence in this group has been ascribed to the use of concomitant medications. Especially the administration of calcineurin inhibitors next to MMF has been shown to influence MPA exposure. In lupus nephritis, MMF treatment is often combined with the use of prednisone. However, there does not seem to be a relationship between glucocorticoid dose and MPA-AUC0-12.

Associations between MPA-AUC0-12 and disease parameters were also investigated in the present study, but the previously reported associations of MPA-AUC0-12 with serum albumin and creatinine clearance could not be confirmed. It should be noted that the findings of previous studies are partly based on a cohort of SLE and ANCA-associated small vessel vasculitis patients together. In addition, it is not the first time that these findings could not be replicated in a group of SLE patients only. This may suggest that there are other variables that influence MPA variability in SLE patients, such as the aforementioned immunological markers or genetic factors which have been reported in renal transplantation patients. Studies with larger cohorts are needed to assess the determinants of variability in MPA exposure in patients with lupus nephritis.
Pharmacokinetic monitoring in MMF therapy has not only been recommended because of the high inter-patient variability in MPA exposure, but also because MPA exposure has been related to clinical outcomes. In patients with autoimmune diseases (including SLE), higher exposure has been associated with lower disease activity and better protection from recurrence of active disease. One study has even suggested that MPA-AUC$_{0-12}$ is a better predictor of renal outcome than clinical or standard laboratory measures in patients with lupus nephritis. In the present study, an individualized dosing regimen with a target MPA-AUC$_{0-12}$ of 60-90 mg*h/l was associated with a good renal outcome after six and 12 months of treatment. The majority of patients were either partial or complete responders and only two patients (12.5%) failed to respond to MMF therapy.

Although pharmacokinetic studies in renal transplantation patients have shown a relationship between high MPA concentrations and the occurrence of adverse events, previous studies which have focused solely on SLE patients did not find a similar association. Also in the present study, adverse events were not related to MPA-AUC$_{0-12}$ levels. Two patients discontinued MMF treatment because of side effects, but one patient only switched after two years of treatment in which complete remission had been achieved. In general, the percentage of patients with adverse effects was low and side effects were well tolerable. This favorable outcome appears to be an additional positive effect of concentration controlled treatment.

Limitations of the present study are the small sample size and the lack of a control group. However, our patient population was homogenous in duration of MMF treatment and the circumstances of MMF initiation (i.e., after six pulses of low dose IVC). This makes the results relevant for SLE patients with proliferative lupus nephritis who are treated with low dose IVC followed by MMF. Of course a randomized controlled trial comparing fixed dose to concentration controlled treatment would be necessary to determine the clinical superiority of an optimized dosing regimen in patients with lupus nephritis with certainty. In addition, the study did not include patients with membranous
lupus nephritis, so that the results only pertain to patients with pure proliferative lupus nephritis.

In conclusion, concentration controlled dose adjustments with a target MPA-AUC$_{0-12}$ of 60-90 mg*h/l resulted in optimized MPA exposure and decreased variability. Moreover, in the context of a modified version of the Euro-Lupus protocol this individualized dosing regimen was associated with an excellent renal outcome at 12 months of follow-up. An optimized dosing regimen through concentration controlled treatment appears to result in a better efficacy and safety profile in lupus nephritis.
REFERENCES


