Mycophenolate mofetil as adjunctive therapy for MMN patients: a randomized, controlled trial


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Multifocal motor neuropathy (MMN) is an immune-mediated disorder characterized by slowly progressive asymmetrical limb weakness. Treatment with immunoglobulins (IVIg) leads to improvement of muscle strength. Anecdotal evidence suggests that immunosuppressive drugs as adjunctive therapy may be beneficial. Mycophenolate mofetil (MMF) is a potent and safe immunosuppressant. Safety and efficacy of MMF as adjunctive therapy for MMN patients receiving IVIg maintenance treatment were evaluated in a randomized controlled trial. MMN patients responding to IVIg treatment were eligible for randomization. Muscle strength and functional status were assessed at monthly intervals for 1 year. Three months after the start of MMF or placebo treatment, IVIg doses were reduced stepwise, until a deterioration of functioning or decline in muscle strength could be observed. An IVIg dose reduction of 50% during adjunctive treatment was defined as a primary endpoint. Secondary outcome measures were improvement in muscle strength and functional status after 3 months and reduction of anti GM1-IgM titres after 12 months of MMF treatment. Twenty-eight patients were randomized. One patient allocated to MMF reached the primary endpoint of 50% IVIg dose reduction. After 12 months IVIg reduction did not differ significantly between the two treatment groups. Patients did not experience drug toxicity and none of the patients showed significant disease progression after 12 months. Muscle strength and functional scores after 3 months and anti GM1-IgM titres after 12 months did not change. Adjunctive treatment of MMN patients with MMF at a dose of 1g twice daily is safe but does not alter disease course or allow significant reduction of IVIg doses.

Keywords: multifocal motor neuropathy; immune-mediated neuropathy; immunosuppressant; treatment; immunoglobulins

Abbreviations: MMN = multifocal motor neuropathy; IVIg = intravenous human immunoglobulins; MMF = mycophenolate mofetil; MMT = manual muscle testing; MRC = Muscle Research Council; SES = self-evaluation scale; ELISA = enzyme-linked immunosorbent assay; SAE = Serious adverse events


Introduction

Multifocal motor neuropathy (MMN) is characterized by slowly progressive, predominantly distal, asymmetrical limb weakness. Age at onset is between 20 and 75 years of age and men are more frequently affected than women (Nobile-Orazio et al., 2005; Van Asseldonk et al., 2005). Motor nerves show conduction block outside the usual sites of nerve compression (Pestronk et al., 1988). The presence of antiglycolipid IgM antibodies and the beneficial effect of immunomodulating therapy suggest that MMN is an immune-mediated neuropathy (Pestronk et al., 1988; Willison and Yuki, 2002; Nobile-Orazio et al., 2005). Placebo-controlled studies showed that treatment with intravenous human immunoglobulins (IVIg) improves muscle strength of patients with MMN (Azulay et al., 1994; Federico et al., 2000; Leger et al., 2001). The beneficial effect of IVIg lasts for several weeks, and repeated IVIg infusions are necessary to maintain muscle strength. Maintenance IVIg treatment is expensive and may not prevent long-term progression of motor deficits and axonal degeneration in MMN (Van den Berg-Vos et al., 2002; Terenghi et al., 2004). Frequent infusions may also be burdensome to patients, but at present there is no therapeutic alternative to IVIg therapy. Plasma exchange...
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is probably ineffective, and prednisone may worsen disease course in some patients (Donaghy et al., 1994; Umapathi et al., 2005). Anecdotal reports suggest that cyclophosphamide may be effective as primary therapy (Pestronk et al., 1988; Feldman et al., 1991) or adjunctive therapy to IVlg, but its toxicity may preclude long-term use in relatively young patients (Baker et al., 1987; Meucci et al., 1997). Evaluation of the efficacy of adjunctive immunosuppressive therapy that would allow reduction of IVlg doses used by MMN patients is needed (Umapathi et al., 2005).

Mycophenolate mofetil (MMF), a morpholinoethyl ester of the active compound mycophenolic acid, is a potent immunosuppressive agent. It blocks purine synthesis in activated T and B lymphocytes and selectively inhibits their proliferation. The efficacy and safety of MMF in preventing rejection of renal transplants, when used in combination with corticosteroids and cyclosporine, has been shown in randomized, double blind trials (Halloran et al., 1997; Allison and Eugui, 2000). It has no major mutagenic effect, nor does it seem to cause organ toxicity (Halloran et al., 1997; Allison and Eugui, 2000). Anecdotal reports and small studies suggest that MMF may be useful in the treatment of immune-mediated neuromuscular diseases, including immune-mediated neuropathies, myositis and myasthenia gravis (Giafaloni et al., 2001; Mowzoon et al., 2001; Chaudhry et al., 2001; Meriggioli et al., 2003a,b; Pisoni et al., 2007; Radziwill et al., 2006). One small open study shows IVlg dose reductions of 50 to 100% in three out of four MMN patients after treatment with MMF while maintaining muscle strength (Benedetti et al., 2004).

We performed a randomized placebo-controlled clinical trial to investigate the efficacy and safety of MMF as adjunctive therapy in patients with MMN on IVlg maintenance treatment.

Patients and methods

Patients

Patients fulfilling the diagnostic criteria for MMN as described previously (Van den Berg-Vos et al., 2000; Hughes, 2001), who experienced clinical improvement after IVlg treatment and who visited the outpatient clinic of the Department for Neuromuscular Diseases of the UMC Utrecht on a regular basis, were invited to participate in the study. Exclusion criteria were (1) severe concurrent medical conditions that might interfere with treatment, (2) pregnancy or (3) age younger than 18 years. The Medical Ethical Committee of the UMC Utrecht approved the protocol prior to the study and all patients gave written informed consent. The procedures were in accordance with the Helsinki declaration (1975, revised 2000).

Study design

This randomized, double-blind, placebo-controlled study was conducted at the Department of Neurology of the UMC Utrecht from June 2004 until October 2005. Patients were randomly assigned to receive either MMF (500 mg twice daily during the first week, 1000 mg twice daily thereafter) or placebo for a period of 12 months. MMF was obtained from Roche Pharmaceuticals, and MMF and placebo were packed in identical capsules. MMF was obtained free from Roche. Roche pharmaceuticals was not involved in the study design and did not support the performance of the trial. All patients received the same brand of immunoglobulins; Gammagard (Baxter). Randomization was performed by one of the investigators (RB), using block randomization with stratification for the extent of muscle weakness at baseline (treatment group A: MRC sum score ≤95; treatment group B: MRC sum score >95). After allocating the patients to one of the treatment groups, each patient was given a number (0–15 to patients allocated to treatment group A, numbers 16–30 to patients allocated to treatment group B). Randomization numbers were passed on to the research pharmacist, who had randomly assigned MMF/placebo to corresponding numbers. Only the research pharmacist had access to the trial codes. Trial medication was packed in blank containers and handed out to the patients. Neither investigators nor participants were aware of group assignment until the end of the trial. Inclusion of 13 patients in both the placebo and MMF group allowed the detection of a 50% reduction of IVlg doses, with a two-sided α-level of 0.05 and a power (1-β) of 80%.

Primary and secondary outcome measures

The primary outcome measure was defined as a reduction in IVlg dose of 50% or more, expressed as the mean IVlg dose per week, after 1 year of MMF/placebo treatment. Secondary outcome measures were improvement in muscle strength and functional status after 3 months of MMF treatment and reduction of anti GM1-IgM titres after 12 months of MMF treatment.

Functional assessments

Clinical evaluation was conducted at baseline and at 4–5 week intervals for a period of 1 year. Muscle strength and functional status were assessed at each visit. Muscle strength was assessed by manual muscle testing (MMT) using the Muscle Research Council (MRC) scale of 15 bilateral muscle groups (shoulder abduction, elbow flexion, elbow extension, wrist flexion, wrist extension, finger flexion, finger spreading and finger extension, hip flexion, knee extension, knee flexion, ankle dorsiflexion and ankle plantar flexion, toe flexion and toe extension). Weakness was defined as an MRC score <5 (Daniels and Worthingham, 1980). MRC sum scores were calculated by summation of all MRC values (maximum score 150). Hand-held dynamometry was performed if there was clinically appreciable weakness (i.e. MRC <5) in muscles for shoulder abduction, elbow flexion, elbow extension, wrist extension, hand grip, hip flexion, knee flexion, knee extension or ankle dorsiflexion (van der Ploeg et al, 1991). Grip strength of the hands was measured by dynamometry. Functional impairment was assessed by (i) Guy’s neurological disability scale, which ranks functional impairment in limbs from 0 (normal functioning) to 5 (impossible to use arm or leg), (ii) the self-evaluation scale (SES) (Leger et al., 2001), which scores 5 motor activities from daily life selected by the patient together with the physician at baseline from 0 (normal functioning) to 5 (impossible to perform activity) and by (iii) the nine hole peg test (Sharrack and Hughes, 1999; Oxford et al., 2003).
IVlg dose reduction
Enrolled patients were treated with MMF or placebo for a period of 3 months prior to stepwise reduction of IVlg doses. All patients received IVlg maintenance treatment at a regular basis varying from every 2 to every 5 weeks. For each patient the mean IVlg dose per week was calculated. IVlg doses were reduced with 5 g per administration if they equalled or exceeded 15 g/week and with 2.5 g per administration if they were lower than 15 g/week. Dose reduction was continued until: (i) patients reported an increase of at least 1 point on the Guy’s Neurological Disability scale score in arms or legs, or an increase of 1 point of at least two items of the SES, or (ii) dynamometry showed a 50% reduction in muscle strength in at least two clinically affected muscles or muscle groups, or (iii) patients reported an unacceptable decline in daily functioning. The IVlg dose was then increased stepwise until muscle strength or functional status improved and stabilized, and a second stepwise reduction of IVlg doses was attempted. After two unsuccessful attempts, IVlg reduction was discontinued. If functional assessments or muscle strength did not improve after dose increase, patients received an IVlg loading dose of 1.2 g/kg in the course of 3 days, after which the IVlg dose used prior to clinical deterioration was continued and no further attempts were made to reduce dosage.

Anti-GMI IgM ELISA
GM1-specific antibody titres were assessed by enzyme-linked immunosorbent assay (ELISA) as described before, with minor modifications (van den Berg et al., 1992). Briefly, 100 µl methanol containing 2.5 µg ganglioside (Calbiochem, San Diego, SA) was added to 96% well plates (NUNC, Maxisorp, Roskilde, Denmark) and left to evaporate overnight at room temperature. Plates were then incubated with 200 µl of a 1% bovine serum albumin (BSA) (Roche Diagnostics, Manheim, Germany) in phosphate buffer saline (PBS, 0.15 M NaCl, 0.01 M NaH₂PO₄, pH 7.4) for 4 h at room temperature. Wells saturated with 1% BSA solution served as controls throughout experiments. Patient sera (diluted 1 : 100 in PBS 1% BSA) were serially diluted in triplicate and incubated overnight at 4°C. After washing 6 times with PBS, peroxidase-conjugated rabbit anti-human IgM (DAKO, Danmark) diluted 1 : 1000 in PBS 1% BSA was added and incubated for 3 h at room temperature. Plates were developed using ABTS® (Roche Diagnostics, Manheim, Germany) as a substrate, and read at 405 nm. Anti-GM1 antibody titres were defined as the dilution yielding an OD of ≥0.10 after subtraction of background values.

Safety
At each visit a questionnaire was completed to document adverse events. Haematological parameters (complete blood count), aminotransferase concentrations, alkaline phosphatase, gamma glutamic transpeptidase, erythrocyte sedimentation rate, serum creatinine, electrolytes and glucose were monitored (at 0, 1, 2, 3, 6, 9 and 12 months). Serious adverse events (SAEs) were reported directly to the Medical Ethical Committee. SAEs were defined as death or severe (life-threatening) infections and haemorrhagic events resulting in hospital admission, neoplasms, events resulting in disabling conditions and drug overdose.

Statistical analysis
Differences in baseline characteristics of placebo- and MMF-treated patients were analysed using a Chi-square test for non-continuous variables and unpaired T-tests for continuous variables. Unpaired T-tests were used to compare differences in IVlg reduction, muscle strength and functional assessments between the two groups at 3 and 12 months as compared to baseline. The paired-sample T-test was used to compare outcome measures at baseline and at 12 months, and to compare GM1-IgM titres at baseline and at the end of the trial. Chi-square test was used for comparison of the distribution of patients with and without GM1-IgM titres, the number of adverse events or abnormal results of ancillary examinations between groups. A P-value of <0.05 was considered significant. All results were analysed on an intention to treat basis.

Results
Patients
Sixty-five patients with MMN were considered for enrolment. Twenty-six patients were excluded because they either did not receive IVlg maintenance treatment (n = 14), received other treatment (n = 8, of which six on interferon β1a and two on immunosuppressive treatment), were lost to follow-up (n = 3) or had died (n = 1). Another 10 patients on maintenance IVlg treatment refused to participate. The remaining 29 patients met the inclusion criteria and were invited to participate. One patient was excluded from randomization after enrolment, because muscle strength fluctuated considerably between control visits despite IVlg treatment. The remaining 28 patients were randomized between June 2004 and September 2004 (Fig. 1). Twenty-four patients fulfilled the clinical criteria for definite MMN, three for probable MMN and one for possible MMN. Two patients withdrew consent after 3 months of trial medication: one patient in the MMF group due to an adverse event (flu-like symptoms) and one patient in the placebo group because she experienced unacceptable deterioration. None of the patients was lost to follow-up. Eleven patients (79%) in the placebo group and 13 (93%) in the MMF group were male. Mean age at disease onset was 38 (SD 9) years in the MMF group and 41 years (SD 12) in the placebo group. At inclusion, mean age in the MMF group was 50 years (SD 8) and 49 years in the placebo group (SD 8). Mean duration of IVlg maintenance treatment was 5.0 years (SD 2.5) in the MMF group and 4.3 years (SD 2.5) in the placebo group. Mean IVlg dose per week in the MMF group was 15.5 g/week (SD 5) and 16.2 g/week (SD 4) in the placebo group. Data on muscle strength and functional scores at baseline are summarized in Table 1. Baseline characteristics did not differ between groups.

Primary outcome measure: IVlg reduction
Mean IVlg doses per week are summarized in Table 1. IVlg dose reduction was started after 3 months of adjunctive
treatment with MMF or placebo. Six months after onset of treatment the mean IVIg dose was reduced to 13.7 g/week in the placebo group and to 13.1 g/week in the MMF group. The lowest dose was 13.1 g/week at 6 months in the placebo group and 12.3 g/week at 7 months in the MMF group. From the seventh month on, mean IVIg doses were gradually increased to 20.2 g/week in the placebo group and 18.0 g/week in the MMF group at 12 months. Mean IVIg doses or dose reduction did not differ significantly between MMF and placebo group at any stage of the study.

Only one patient in the MMF group fulfilled the primary outcome measure: in this patient the dose was reduced by 52% from 21 g/week at baseline to 10 g/week at 12 months. One year after stopping MMF treatment in this patient, the IVIg dose was gradually increased to 13.3 g/week to maintain muscle strength.

At 12 months of treatment, mean IVIg doses per week were higher compared to baseline in placebo and MMF groups due to reloading of IVIg to restore deterioration of muscle strength and functional scores. Six months after the trial had ended, mean IVIg doses had returned to baseline levels (17.0 g/week for both groups).

Secondary outcome measures: muscle strength, functional status, anti-GM1 antibody titres

MMF treatment adjunctive to IVIg during the first 3 months of the trial did not lead to improved muscle strength or functional status (Table 1): muscle strength measured by MMT and dynamometry (data not shown) did not change significantly nor did any of the functional impairment scores after 3 months. Muscle strength and functional impairment scores were not significantly different between placebo and MMF groups at any stage during the trial.

At baseline anti-GM1-IgM-titres in sera from eight (57%) patients from the MMF group and eight patients (57%) from the placebo group could be detected. Anti-GM1-IgM titres in serum samples from 23 patients (12 patients from the MMF group, 11 patients from the placebo group) obtained at inclusion and at the end of the trial were determined. GM1-IgM titres were similar at baseline and at 12 months in placebo and MMF groups ($P = 0.40$).

### Adverse events and side-effects

Side-effects are listed in Table 2. Abnormal ancillary investigations (haematological, hepatic or renal function) were not observed. Headache occurred significantly more often in the MMF group. Gastrointestinal complaints, sleep disturbance and rash were more frequently reported by patients treated with MMF, but this failed to reach statistical significance. One patient from the MMF group was diagnosed with cutaneous lupus erythematosus during the study. The consulting dermatologist concluded that a relation of lupus erythematosus onset and the use

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**Table 1** Effect of MMF as adjunctive treatment in patients on IVIg maintenance treatment

<table>
<thead>
<tr>
<th>Period</th>
<th>Inclusion</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
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</thead>
<tbody>
<tr>
<td>IVIg dose (g/week)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMF</td>
<td>15.5 (5)</td>
<td>15.5 (5)</td>
<td>13.7 (5.5)</td>
<td>18.0 (10)</td>
</tr>
<tr>
<td>Placebo</td>
<td>16.2 (4)</td>
<td>17.2 (7)</td>
<td>13.1 (4.0)</td>
<td>20.2 (11)</td>
</tr>
<tr>
<td>MRC sum score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMF</td>
<td>164 (13)</td>
<td>162 (15)</td>
<td>162 (15)</td>
<td>160 (17)</td>
</tr>
<tr>
<td>Placebo</td>
<td>161 (13)</td>
<td>163 (12)</td>
<td>160 (13)</td>
<td>163 (11)</td>
</tr>
<tr>
<td>SES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMF</td>
<td>2.0 (0.7)</td>
<td>2.0 (0.6)</td>
<td>1.6 (0.7)</td>
<td>2.1 (0.7)</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.8 (0.8)</td>
<td>1.7 (0.8)</td>
<td>2.0 (0.6)</td>
<td>1.7 (0.7)</td>
</tr>
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</table>

**Guy’s neurological disability scale**

<table>
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<tbody>
<tr>
<td>2.4 (1.9)</td>
<td>2.9 (1.7)</td>
</tr>
<tr>
<td>2.9 (1.6)</td>
<td>2.9 (1.5)</td>
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**9HPT right hand (s)**

<table>
<thead>
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<th>MMF</th>
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</thead>
<tbody>
<tr>
<td>24 (9)</td>
<td>22 (5)</td>
</tr>
<tr>
<td>28 (10)</td>
<td>31 (15)</td>
</tr>
</tbody>
</table>

**9HPT left hand (s)**

<table>
<thead>
<tr>
<th>MMF</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 (15)</td>
<td>28 (16)</td>
</tr>
<tr>
<td>22 (4)</td>
<td>22 (4)</td>
</tr>
</tbody>
</table>

Note: Data are mean (SD). MMF = mycophenolate mofetil; IVIg = intravenous immunoglobulins; SES = self-evaluation scale; 9HPT = nine hole peg test.
The design of this study was similar to ours but patients were treated and the effect measured in an open fashion. In our randomized, double-blind, placebo-controlled trial in 28 patients with MMN, IVIg doses of only one patient could be reduced by 50%. In addition, no trend towards a significant effect of MMF was found in any of the outcome measures.

Since MMN and many other neuromuscular disorders are relatively rare, conducting a placebo-controlled trial may be cumbersome and non-evidence-based experimental treatment tempting. The outcome of this randomized controlled clinical trial on the efficacy of MMF in MMN does not support the results of an uncontrolled preliminary study, and demonstrates the necessity of performing placebo-controlled trials in neuromuscular disease. MMF is currently being studied in two prospective, randomized, double-blind, placebo-controlled trials to better establish its role in the treatment of myasthenia gravis.

Add-on therapy that allows IVIg dose reduction in MMN would reduce health care costs, and the patient burden of repeated infusions. This randomized controlled trial was designed to allow an IVIg dose reduction of 50%. Although we cannot exclude the possibility that MMF would allow IVIg dose reduction of less than 50%, we feel that only significant reductions, or an additional beneficial effect on disease course would justify the lifelong use of immunosuppressive drugs (Vernino et al., 2005; Finelli et al., 2006). Moreover, there was no trend towards lower IVIg doses in patients allocated to MMF as compared to placebo.

Treatment of kidney transplant patients with 1000 mg of MMF twice daily effectively suppresses B-cell and T-cell proliferation, and has relatively few side-effects (Allison and Eugui, 2000). Steady-state MMF plasma levels are reached within 90 days after the start of treatment (Hale et al., 1998), and beneficial effects on disease course of autoimmune disorders are observed after a median treatment period of 11 weeks (Ciafaloni et al., 2001; Meriggioli et al., 2003b). It, therefore, seems unlikely that MMF treatment of MMN patients prior to reducing IVIg doses was too short to detect a beneficial effect of the combined treatment of IVIg and MMF on muscle strength or functional status. In addition, it seems unlikely that reduction of IVIg maintenance treatment was started before MMF was effective. Patients were treated with 1000 mg of MMF twice daily, because this dose induces plasma levels well above the minimum required plasma concentration in patients without immunosuppressant co-medication and with normal renal function (Halloran et al., 1997; Hale et al., 1998; van Hest et al., 2006).

The use of MMF did not reduce GM1-specific IgM titres in plasma of MMN patients. Uncontrolled studies showed that treatment of MMN patients with cyclophosphamide reduces GM1-specific IgM titres (Pestronk et al., 1989). Similarly, two studies suggested that treatment with MMF, 1000 mg twice daily, is associated with a reduction of acetylcholine receptor-specific antibody titres in plasma.
from myasthenia gravis patients (Ciafaloni et al., 2001; Meriggioli et al., 2003b). It is not exactly clear why MMF does not suppress the function of GM1-specific B-cells and plasma cells. Glycolipid-specific B-cells may have lower proliferation rates than protein-specific B-cells, and may be less susceptible to the proliferation suppressing effects of MMN.

Clarification of MMN pathogenesis may facilitate the selection of immunosuppressive drugs which may have a beneficial effect on disease course. Cost-effectiveness should be balanced against the risks of short- and long-term toxicity. Future randomized, placebo-controlled trials in MMN may focus on efficacy of other immunosuppressive (e.g. azathioprine, methotrexate, cyclophosphamide or cyclosporine) or immunomodulating (e.g. rituximab or interferon (1a) drugs.

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References
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