Mycophenolate mofetil versus cyclophosphamide for inducing remission of ANCA vasculitis with moderate renal involvement

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Abstract

Objective. We performed a single-centre non-blinded clinical trial to compare the clinical efficacies of mycophenolate mofetil (MMF) and intermittent cyclophosphamide (CTX) pulse therapy as induction treatments in patients with antineutrophil cytoplasmic antibody (ANCA) vasculitis (AAV) and moderate renal involvement.

Methods. Patients with active AAV and serum creatinine <500 µmol/L received either MMF treatment (MMF group) or monthly CTX pulse therapy (CTX group) for 6 months. Disease activity was assessed using the Birmingham Vasculitis Activity Score (BVAS). The disease activity, remission rate, renal function and adverse reactions were compared between the two groups.

Results. A total of 35 patients (15 male, 20 female: aged 49.1 ± 12.2 years) were enrolled, with 18 in the MMF group and 17 in the CTX group. Of the 35 patients, 28 were MPO-ANCA positive and 2 were PR3-ANCA positive. Four patients were lost to follow-up in the CTX group. At Month 6, BVAS scores were much lower in the MMF group than in the CTX group (0.2 ± 0.89 versus 2.6 ± 1.7, P < 0.05). In the intent-to-treatment analysis, 14 of 18 patients (77.8%) treated with MMF and 8 of 17 patients receiving CTX (47.1%) had complete remission with an absolute difference of 30.7%. Eight of 18 patients (44.4%) in the MMF group and 2 of 17 patients (15.4%) in the CTX group recovered renal function. Serum ANCA decreased to normal in 41.7% of patients in the MMF group and in 16.7% in the CTX group. Side effects in the MMF group were pneumonia (1), herpes zoster (1) and gastrointestinal symptoms (2), and in the CTX group were leukocytopenia (1), gastrointestinal distress (4) and pneumonia (1).

Conclusion. Our study suggests that MMF effectively ameliorates disease activity and considerably improves renal function in patients with AAV. Further large-scale multicentre prospective randomized controlled trials will be needed to confirm these findings.

Keywords: antineutrophil cytoplasmic antibody; cyclophosphamide; mycophenolate mofetil; vasculitis

Introduction

The introduction of cyclophosphamide (CTX) in combination with corticosteroid for the induction of remission of antineutrophil cytoplasmic antibody (ANCA) vasculitis (AAV) has markedly improved the outcome of patients with AAV. Although most AAV patients achieve remission or partial remission [1–2], the relapse rate is still high. In fact, long-term remission rates after induction treatment with CTX and steroid were only 60–85%, and long-term survival rates were not ideal. For patients with severe renal damage, the 1-year mortality rate was still as high as 25%. Even for patients not dialysis dependent upon diagnosis, the 5-year survival rate was only about 70% [3]. Survival is even lower in Chinese patients with microscopic polyangiitis (MPA), who show 1-year patient and renal survival rates of only ∼70% [4]. Moreover, a high risk of side effects due to large doses of CTX was associated with poor long-term prognosis. Therefore, it is imperative to seek more effective induction and maintenance therapies to improve long-term outcomes.

In recent years, mycophenolate mofetil (MMF) has been widely used in the treatment of severe immune-mediated glomerular nephritis. Clinical studies have revealed that MMF is more effective than CTX for treating lupus nephritis with vasculitic lesions [5,6]. Previous non-controlled clinical observations also suggested that MMF was effective for attenuating vasculitis-induced renal damage and improving renal function [7,8]. In addition, MMF was found to be effective and safe for maintenance treatment of AAV [9,10]. Nevertheless, there have been no controlled trials that evaluated the effects of MMF during induction treatment for AAV. The present study aimed to compare the effects of MMF and CTX during induction treatment for AAV with renal damage.

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Patients and methods

Patient selection

Patients with newly diagnosed active AAV were recruited between June 2003 and December 2004 at the Research Institute of Nephropathy at Jinling Hospital of Nanjing University School of Medicine. AAV and clinical variants such as microscopic polyangiitis (MPA) or Wegener granulomatosis (WG) were diagnosed in accordance with the criteria from the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitides [11]. All patients were older than 18 years and presented with renal involvement that manifested as either haematuria, red cell casts with serum creatinine (SCr) <500 μmol/L, or both disorders. Although most patients were positive for serum ANCA, a few were included with renal biopsy-confirmed pauci-immune segmental necrotizing and crescent glomerulonephritis, but with negative serum ANCA.

Patients with the following conditions were excluded from the study: (a) large dose cytotoxic drug treatment for 6 months prior to the study; (b) severe viral infection (HBV, HCV and CMV) or known HIV infection; (c) acquired immune deficiency; (d) severe renal failure, with SCr ≥500 μmol/L, or renal replacement treatment for more than 2 weeks; (e) life-threatening organ manifestation (lum haemorrhage, central nervous system involvement); (f) active tuberculosis; (g) liver dysfunction measured on at least two separate occasions; (h) pregnancy or inadequate contraception if female and (i) age under 18 years or over 65 years.

The study was approved by the local ethics committees and all patients gave written informed consent.

Study design

Patients entered into the study were non-blinded and were randomly divided into two groups: one group received MMF therapy in combination with steroids (MMF group), and the other received CTX therapy in combination with steroids (CTX group). The observation period was 6 months.

Drug regimens

Patients in both groups initially received intravenous methylprednisolone pulse therapy (0.5 g, once daily, for three consecutive days), followed by oral prednisone at a dose of 0.6–0.8 mg/(kg day) for 4 weeks, which was then tapered by 5 mg each week to 10 mg/day.

In the MMF group, MMF was given as 2.0 or 1.5 g/day (for body weight <50 kg) for 6 months. The dose of MMF was reduced or withdrawn if the following side effects occurred: (a) infection (for mild infection, MMF was continued, but the dose was reduced by half; for severe infection, the drug was discontinued); (b) leucocytopenia (for white blood cell (WBC) count <3000/μL, MMF was reduced by half; for WBC count <2000/μL, MMF was withdrawn) and (c) gastrointestinal symptoms (MMF was reduced or withdrawn if severe vomiting or diarrhoea occurred). Patients were withdrawn from the study if MMF was discontinued for more than 1 month.

In the CTX group, intravenous CTX was given for 6 months as 0.75–1.0 g/m² body surface area in monthly pulses according to a National Institutes of Health (NIH) protocol. Dosage was modified if nadir white-cell counts reached 2500 cells or less at 7 to 10 days after the infusion. The patients were adequately hydrated after CTX pulse infusions.

No other immunosuppressive drugs were given in either group. For patients with hypertension, a calcium channel blocker was administered to maintain blood pressure near 130/80 mmHg. Angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists were continued if they had been used for more than 4 weeks before entering the trial. Otherwise, these drugs were not allowed for use during the observation period.

Renal pathology

Percutaneous renal biopsies were taken from all patients prior to the study. Renal tissues were stained with HE, PAS, PASM and Masson for light microscopy examination. Frozen sections were tested for immunoglobulins and complement deposition by direct immunofluorescence (IF).

Serum ANCA

ANCA titers were assessed by indirect (IIF) on ethanol-fixed human neutrophils (EUROIMMUN, Germany) and by antigen-specific ELISA. Purified PR3 and MPO were used as solid-phase ligands in ELISA.

Clinical assessment

The patients were monitored each month. Routine blood work, serum albumin, SCr, liver enzymes, serum ANCA, CRP, 24-h urine protein, urinary red blood cell counts and vasculitis activity scores were evaluated. Vasculitis activity was assessed by the Birmingham Vasculitis Activity Score (BVAS) [12] method. BVAS scores included BVAS1 (which refers to new or worse symptoms due to vasculitis activity, with higher scores indicating more active disease) and BVAS2 (which refers to persistent symptoms due to vasculitis activity).

The primary measure for efficacy was the remission rate at 6 months. Remission was defined as no clinical signs of vasculitis, improved or stable renal function, no active urine sediments and BVAS1 = 0, BVAS2 < 1 [2,11]. The secondary measure was any changes in renal function as well as side effects.

Statistical analysis

All data were expressed as means ± standard deviation or as constituent ratios. Chi-square tests were performed with measurement data, and two-tailed t-tests were performed with enumeration data. In our primary analysis, patients lost to follow-up were marked as non-responders. We analysed data only from patients for whom outcome information was available. Comparisons of area under the curve for BVAS in the two groups were performed using t-tests after
log-transformation. A value of $P < 0.05$ was considered statistically significant.

**Results**

**Baseline condition**

A total of 35 patients (15 males, 20 females), ages ranging from 18 to 71 years (mean $49.1 \pm 12.2$ years), were enrolled in the study. Eighteen patients were included in the MMF group and 17 patients were in the CTX group. Among these patients, 30 (85.7%) were ANCA positive (28 MPO-ANCA, 2 PR3-ANCA) and 5 were ANCA negative. All had MPA except one patient that was diagnosed with WG.

There were no differences between the two groups in clinical or renal pathological characteristics (Tables 1 and 2). A high incidence of necrotizing arteriolitis (Figure 1) was found in both groups.

Four patients in the CTX group were lost to follow-up after 3 months of treatment without any improvement in clinical activity, while none in the MMF group were lost to follow-up.

**Clinical efficacy and disease activity**

After 6 months of treatment, BVAS scores were decreased in both groups (Figure 2), and these reductions were greater in the MMF group than in the CTX group (at 3 months:

**Table 2. Baseline renal histological characteristics**

<table>
<thead>
<tr>
<th></th>
<th>MMF group $(n = 18)$</th>
<th>CTX group $(n = 17)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total crescents (%)</td>
<td>54.3 ± 27.0</td>
<td>56.6 ± 34.4</td>
</tr>
<tr>
<td>Glomerular necrosis, n (%)</td>
<td>14 (77.8)</td>
<td>14 (82.3)</td>
</tr>
<tr>
<td>Globule sclerosis (%)</td>
<td>15.5 ± 13.5</td>
<td>16.4 ± 13.5</td>
</tr>
<tr>
<td>Segmental sclerosis (%)</td>
<td>15.3 ± 10.2</td>
<td>4.2 ± 3.9</td>
</tr>
<tr>
<td>Necrotizing arteriolitis, n (%)</td>
<td>11 (61.1)</td>
<td>10 (58.8)</td>
</tr>
</tbody>
</table>

Fig. 2. BVAS score after the treatment showing that BVAS score decreased more significantly in the MMF group.
Fig. 3. Mean SCr level after MMF or CTX treatment.

1.76 ± 2.56 versus 3.54 ± 1.88; at 6 months: 0.2 ± 0.89 versus 2.6 ± 1.7, $P < 0.05$).

In the intention-to-treat analysis, assuming that those lost to follow-up did not show an early response, MMF therapy resulted in a rapid response and high remission rate. At 6 months, 14 of 18 patients in the MMF group (77.8%) and 8 of 17 patients in the CTX group (47.1%) experienced remission, yielding an absolute remission difference of 30.7%.

When patients lost to follow-up were excluded from the analysis, 14 (77.8%) of 18 in the MMF group had remission, and 8 (61.5%) of 13 in the CTX group had remission after 6 months of treatment, creating a difference of 16.3%.

**Renal function**

Changes in mean SCr in the two groups are shown in Figure 3. Although mean SCr was lower in the MMF group than in the CTX group during follow-up, this difference did not reach statistical significance. At Month 6, significantly more patients with normal renal function (SCr $\leq 1.5$ mg/dL) were observed in the MMF group than in the CTX group (44.4 versus 15.4%, $P < 0.05$).

**Serum ANCA**

During follow-up, changes in serum ANCA were monitored in 17 patients (12 in the MMF group and 5 in the CTX group). After MMF treatment, ANCA levels were undetectable in five patients (41.7%), decreased in six (50%) and did not change in one patient who failed to attain remission.

In the CTX group, ANCA levels were undetectable in one patient (20.0%), decreased in two (40%), and did not change in two (40%) patients.

**Adverse events**

There were no deaths in either group during the 6 months of induction treatment. Adverse events were found in 4 of 18 patients in the MMF group and in 6 of 17 (35.3%) in the CTX group. These events were not different between the two groups (Table 3).

**Discussion**

Mycophenolate mofetil (MMF) selectively inhibits the proliferation of T- and B-lymphocytes by inhibiting purine metabolism. In addition, recent studies suggest that mycophenolic acid (MPA), the active metabolite of MMF in vivo, can significantly suppress the expression of endothelial adhesion molecules and inhibit the adhesion of leukocytes to endothelial cells [13,14], which is a key process in the development of ANCA-associated vasculitis. Clinical studies have shown that MMF effectively reverses acute vascular rejection in renal transplantation [15] and ameliorates lupus vasculitic lesions [5]. These findings provide rational basis for the use of MMF as induction treatment for AAV. We previously reported that three patients with pauci-immune crescentic glomerulonephritis (renal-limited small vasculitis) were successfully treated with MMF [7]. Furthermore, Waiser et al. [8] showed that a patient with relapsed Wegener's granulomatosis (WG), who was intolerant to CTX, was also successfully controlled by MMF. Moreover, a recent uncontrolled clinical study by Joy et al. [16] provided further evidence on the effectiveness of MMF in the treatment of systemic small vasculitis. In their study, 12 patients with refractory AAV (six failed, six relapsed) were switched to MMF induction therapy, and this caused marked decreases in BVAS scores from $9.1 \pm 3.5$ to $2.8 \pm 1.9$ at week 24 and to $2.8 \pm 4.3$ at week 52, with remission rates of 60% at month 6. Taken together, these preliminary clinical studies indicate that MMF may provide effective treatment for systemic small AAV. However, controlled studies using MMF have not yet been reported.

Many studies suggest that adjunctive plasmapheresis or immunoadsorption treatment is necessary for patients with dialysis requirements or with pulmonary haemorrhage to improve renal and patient survival [17–19]. We therefore excluded from our study patients with advanced renal failure (SCr $\geq 500$ µmol/L) or with life-threatening active vasculitis (for example, severe pulmonary haemorrhage or central nervous system involvement). The present dose of MMF was taken from clinical studies treating lupus nephritis, and we also considered age, overall condition and immunological status of the patients (lymphocytes and IgG level). Most of the patients received MMF in a dose of 1.5 g/day. The drug was well tolerated and only a few cases were complicated with severe gastrointestinal symptoms and infection (5.6%). In contrast, more patients in the CTX group had complications.
group developed gastrointestinal symptoms and were lost to follow-up.

The main purpose of the present study was to compare the clinical efficacies of the 6-month MMF and CTX treatment on inducing remission and improving renal function in patients with AAV. We found that vasculitic activity scores (BVAS) decreased significantly in both groups during the observation period, but BVAS scores decreased more rapidly and more patients went into remission after 3 months in the MMF group than in the CTX group. At Month 6, remission rates in the MMF group were still higher than in the CTX group (77.8 versus 47.1%). These results suggest that MMF therapy may produce a more rapid response and higher clinical efficacy in controlling vasculitis activity than CTX. More importantly, we found that more patients with MMF than CTX experienced renal function recovery (44.4 versus 15.4%, \( P < 0.05 \)) and either decreases or normalization of serum ANCA (91.7 versus 60%). Many studies have shown that renal function following treatment is an important factor for the long-term renal survival [4,20], and persistently positive ANCA levels following induction therapy represent a great risk factor for relapse [21]. In this context, the findings from the present study strongly suggest that MMF treatment in patients with AAV may significantly improve the long-term renal survival and reduce the rate of relapse. There were two major limitations in this study: one was the small number of the patients studied, and the other was the short observation period. Therefore, prospective multicentre trials with larger numbers of patients are warranted to explore the effect of MMF therapy on long-term renal survival.

We observed that remission rates after MMF or CTX were much lower than in previous studies conducted in various Western countries [1,2,22–23] in which remission was achieved in over 90% of patients. For example, Jayne et al. [2] recently reported that oral CTX induction treatment produced a high AAV remission rate of 77% at 3 months and 93% at 6 months. Several possible factors, including genetic background, ANCA patterns and organ involvement of the patients, may explain the different remission rates among the studies.

Recent evidence from AAV studies suggests that genetic background, environmental factors and pathogenic mechanisms may differ between Far-East and Western populations. In Western populations, positive PR3-ANCA or WG was the predominant pattern in patients with AAV [2,22–24]. In contrast, most of the patients in our study were MPO-ANCA positive. In fact, recent studies indicated that MPO-ANCA-positive vasculitis and clinical forms of MPA were more prevalent in Asian countries including China and Japan. Our data showed that, in 112 patients with AAV, 98 (87.5%) were MPO-ANCA positive, only 13 (11.6%) were PR3-ANCA and 1 (0.9%) was MPO-ANCA and PR3-ANCA double positive (unpublished data). In agreement with this, a predominance of MPO-ANCA patterns and clinical forms of MPA were found in another study from northern China, in which 79.1% were MPA and only 20.4% of the patients were WG [25]. In that same study, patients positive for MPO-ANCA were more prevalent than for PR3-ANCA (60.7% versus 38.2%), even in patients with WG [26]. The proportion of patients positive for PR3-ANCA was also extremely low in Japan. An epidemiological survey in Japan [27] showed that 91% of patients with vasculitis were also MPO-ANCA positive, and none were PR3-ANCA positive or had WG.

Several studies have demonstrated an influence of ANCA pattern and organ involvement on disease manifestation and outcome, although results have been inconsistent. A higher prevalence of SCR was found in Chinese patients with MPO-ANCA than in those with PR3-ANCA (81.5 versus 61.8%) [25]. This is in agreement with Vizjak et al. [28], who found that patients positive for MPO-ANCA had a longer duration of renal disease, a more prominent diffuse glomerular crescent formation and more chronic lesions than patients positive for PR3-ANCA. Furthermore, an additional study [24] found that both active and chronic renal lesions were more common in MPO-ANCA-positive patients than in PR3-ANCA-positive patients. Renal involvement was regarded as an important factor for the poor response to treatment and outcome. In this context, several previous trials have studied patients without renal involvement [2,22,23]. In the present study, the kidney was the main involved organ, and SCR and urine sediments were the main parameters used to assess vasculitic activity and to define the response to treatment. All of the patients had severe renal damage with a mean SCR of 3.56 ± 1.40 mg/dL at the beginning of the study, and renal biopsy revealed diffuse crescent formation, glomerular necrosis and sclerotic lesions. Extraglomerular vasculitis, which is considered to be resistant to various treatments, was also more prominent in our patients (60%) than in previously published studies (4% in EUVAS, 25% in Japan). Collectively, the clinical and histological features of MPO-ANCA-associated vasculitis in our patients may explain the low remission rate in our study. In addition, the greater number of patients lost to follow-up may also explain the total remission rate in the CTX group.

In conclusion, we found that MMF was effective in controlling disease activity and improving renal function in Chinese patients with MPO-ANCA-associated vasculitis. Multicentre clinical trials with long-term follow-up will be necessary to confirm our findings and to extend the observations to PR3-ANCA-positive patients.

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Conflict of interest statement. None declared.

References

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