Mycophenolate mofetil or tacrolimus compared with intravenous cyclophosphamide in the induction treatment for active lupus nephritis

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Abstract

Background. Although the use of aggressive immunosuppression has improved both patient and renal survival of patients with lupus nephritis (LN), the optimal treatment of LN remains challenging. The objective of this study is to assess the efficacy and safety of mycophenolate mofetil (MMF) and tacrolimus compared with intravenous cyclophosphamide (IVC) as induction therapies for active lupus nephritis (ALN).

Methods. In this open-label, 24-week prospective study, 60 patients with biopsy-proven ALN (Classes III, IV, V or combination) were randomly assigned to receive MMF, tacrolimus or IVC in combination with corticosteroids. The remission of proteinuria, systemic lupus erythematosus disease active index and adverse events were compared.

Results. The response rates at 24 weeks were 70% (14/20) in the MMF group, 75% (15/20) in the tacrolimus group and 60% (12/20) in the IVC group (P > 0.05). The complete remission rates were also similar in the three groups (40, 45 and 30%, respectively; P > 0.05). There were more cases of infection in the IVC group (8/20) and the MMF group (8/20) than the tacrolimus group (3/20) and more hyperglycemia in the tacrolimus group (5/20) than the other two groups (2 or 3/20), but the results were not statistically significant among the three groups. Proteinuria decreased and serum albumin increased more quickly in the patients treated with tacrolimus (P = 0.0051 and P = 0.048).

Conclusions. This pilot study suggests that both MMF and tacrolimus are possible alternatives to IVC as induction therapies for ALN in Chinese patients. Tacrolimus possibly results in a faster resolution of proteinuria and hypoalbuminemia. Further studies are necessary to determine the optimal dosage and duration of the therapies.

Keywords: induction therapy; lupus nephritis; mycophenolate mofetil; tacrolimus

Introduction

Advances in immunosuppressive treatment over the past decades have resulted in improved remission rate and long-term renal survival for lupus nephritis (LN) [1, 2]. Glucocorticoids (GC) and cyclophosphamide (CYC) are commonly considered as the standard therapy regimens for remission induction [3]. However, CYC is associated with significant toxicity, particularly infections, malignancy and infertility. Moreover, not all LN patients experience a satisfactory response. Safer, more effective alternative therapies have been sought. Mycophenolate mofetil (MMF) and tacrolimus may be such alternatives.

MMF, a lymphocyte selective antiproliferative agent which was first introduced into clinical use over a decade ago for preventing solid organ transplant rejection, has emerged as an effective and safe immunosuppressive agent. The use of MMF in proliferative LN has been repeatedly tested in a number of controlled trials [4–8]. A consistent observation in many clinical studies is that treatment with MMF is generally well tolerated. Is MMF really better than CYC? The answer remains controversial.

Tacrolimus is a T-cell-specific calcineurin inhibitor that prevents activation of T helper cells, thereby inhibiting transcription of the early activation genes of interleukin (IL)-2 and suppressing the production through T-cell activation of tumor necrosis factor-α, IL-1β and IL-6 [9, 10]. Compared with cyclosporine, it seems more prospective in prevention of transplant rejection and is associated with less hypertensive and adverse cosmetic effects. Early anecdotal experiences suggest that tacrolimus could be a treatment option for patients with lupus, and topical tacrolimus has been used in the treatment of lupus dermopathy [11]. However, the side effects of tacrolimus such as diabetogenesis and nephrotoxicity remain valid concerns. Little evidence of randomized controlled trials has been published on the efficacy and safety when administered with corticosteroids treatment of LN.

So far, there is no report comparing the therapy of MMF, tacrolimus and intravenous cyclophosphamide (IVC) within a single study of LN. In the present study, the efficacy, safety and tolerability of oral MMF plus GC and oral tacrolimus plus GC were compared with those of IVC plus GC for inducing remission of active lupus nephritis (ALN). We hope that it may provide clues for LN therapy regimens selection and other future studies.
Materials and methods

Patients

Patients, aged from 18 to 65 years, were enrolled when meeting the following inclusion criteria: (i) diagnosed as systemic lupus erythematosus (SLE) defined by the 1997 revised American Rheumatism Association criteria, (ii) renal biopsy within 6 months before randomization; showing ALN (Classes III, IV-S or IV-G, V, V + III or V + IV according to the 2003 International Society of Nephrology/Renal Pathology Society classification, chronic index ≤3) and (iii) urinary protein excretion of ≥1.0 g/24 h or above, and/or a recent deterioration in renal function. All the patients were included after providing informed consent. The study was approved by the institutional Ethics Committees, and the registration number in the Chinese Clinical Trial Registry is ChiCTR-TRC-10000896. Reasons for exclusion were treatment with MMF, tacrolimus, cyclosporine or CYC within the previous year, serum creatinine concentration >5.0 mg/dL (442 μmol/L), life-threatening complications such as cerebral lupus, pancreatitis, gastrointestinal hemorrhage within 6 months or active peptic ulcer within 3 months, severe infection, severe cardiovascular disease, bone marrow insufficiency with cytopenia not attributable to SLE or poor drug compliance. Pulse intravenous corticosteroids were prohibited throughout the study.

Treatment regimen

The patients were randomly assigned to one of the three treatment groups (MMF group, tacrolimus group and IVC group) in an open-label manner. Oral MMF was given twice daily, at a dose of 1.5 g/day in patients weighing ≤55 kg and 2.0 g/day in whose body weight >55 kg. The initial dose of tacrolimus was 0.08–0.1 mg/kg/day administered orally in two divided doses and was titrated to maintain 12-h trough levels at <0.3 g/24 h, with normal urine sediment, serum albumin concentration >35 g/L and stabilization (±15%) or improvement in serum creatinine at 24 weeks. Partial remission (PR) was defined as urinary protein excretion between 0.3 and 2.9 g/24 h, having decreased by at least 50% from baseline values, with a serum albumin concentration of at least 30 g/L and relative stabilization (±30%) in serum creatinine.

Statistical analysis

CR or PR at 24 weeks was the primary endpoint. Predefined secondary endpoints included urinary protein excretion, serum albumin concentration, serum creatinine levels, the systemic lupus erythematosus disease activity index (SLEDAI), blood and urine parameters, anti-dsDNA antibody-binding levels, serum C3 concentration, side effects and death. All results were expressed as mean ± SD for normally distributed data, median and range for skewed data and frequency (%) for categorical data. The distribution of demographic, clinical and laboratory attributes among the treatment groups were evaluated by Kruskal–Wallis test, Wilcoxon rank test or t-test. Comparisons of percentages between groups were made with the chi-square test ($\chi^2$) or Fisher exact test, as appropriate.

Results

Patient characteristics

Sixty patients were randomly assigned to receive MMF, tacrolimus or CYC in combination with corticosteroids. Clinical and pathological characteristics in the three groups were similar at baseline (Table 1).

Response to treatments

During the follow-up period, four patients in the MMF group left the study, one withdrew after 8 weeks because of cytomegalovirus infection, two withdrew after 12 weeks because of gastroenteritis and pneumonia, respectively, and one died because of pneumonia at 16 weeks; in the tacrolimus group, one patient withdrew at 8 weeks because of a doubling of serum creatinine level, one withdrew at 16 weeks thereafter until completion of the 24-week induction phase. Patients were withdrawn when they had a doubling of serum creatinine or required dose stoppage for >7 days during the induction phase.

Table 1. Characteristics of the patients at study entry by treatment groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MMF (n = 20)</th>
<th>Tacrolimus (n = 20)</th>
<th>CYC (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 (13.3)</td>
<td>3 (13.3)</td>
<td>2 (10.0)</td>
<td>0.860</td>
</tr>
<tr>
<td>Female</td>
<td>17 (86.7)</td>
<td>17 (86.7)</td>
<td>18 (90.0)</td>
<td>0.860</td>
</tr>
<tr>
<td>Age at enrollment [year; median (range)]</td>
<td>26.5 (16–62)</td>
<td>29 (17–50)</td>
<td>33 (17–64)</td>
<td>0.613</td>
</tr>
<tr>
<td>SLEDAI (mean ± SD)</td>
<td>18.2 ± 4.5</td>
<td>18.3 ± 5.1</td>
<td>18.9 ± 4.9</td>
<td>0.827</td>
</tr>
<tr>
<td>Duration of LN [months; median (range)]</td>
<td>3 (1–120)</td>
<td>3 (1–48)</td>
<td>2 (1–240)</td>
<td>0.608</td>
</tr>
<tr>
<td>Urine protein [g/24 h; median (range)]</td>
<td>3.66 (0.38–9.87)</td>
<td>3.36 (0.5–7.90)</td>
<td>3.70 (0.48–6.44)</td>
<td>0.874</td>
</tr>
<tr>
<td>Serum albumin [g/L; mean ± SD]</td>
<td>12.3 ± 2.8</td>
<td>13.6 ± 3.2</td>
<td>14.0 ± 3.6</td>
<td>0.791</td>
</tr>
<tr>
<td>Serum creatinine [μmol/L; median (range)]</td>
<td>60.50 (39–274)</td>
<td>83.5 (50–423)</td>
<td>87.0 (34–394)</td>
<td>0.232</td>
</tr>
<tr>
<td>eCcr (mL/min/1.73m²; mean ± SD)</td>
<td>96.3 ± 42.4</td>
<td>82.2 ± 28.0</td>
<td>80.6 ± 32.1</td>
<td>0.296</td>
</tr>
<tr>
<td>Renal biopsy class [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III/IV</td>
<td>14 (70)</td>
<td>13 (65)</td>
<td>13 (65)</td>
<td>0.940</td>
</tr>
<tr>
<td>III + V/IV + V</td>
<td>3 (15)</td>
<td>5 (25)</td>
<td>4 (20)</td>
<td>0.940</td>
</tr>
<tr>
<td>V only</td>
<td>3 (15)</td>
<td>2 (10)</td>
<td>3 (15)</td>
<td>0.940</td>
</tr>
<tr>
<td>Anti-dsDNA positive [n (%)]</td>
<td>15 (75)</td>
<td>17 (85)</td>
<td>15 (75)</td>
<td>0.732</td>
</tr>
<tr>
<td>Low C3 complement [n (%)]</td>
<td>16 (80)</td>
<td>17 (85)</td>
<td>17 (85)</td>
<td>0.938</td>
</tr>
</tbody>
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aDAI, disease active index; eCcr, estimated creatinine clearance rate.
weeks because of severe leucopenia and one died because of pneumonia at 12 weeks; in the IVC group, one patient withdrew at 4 weeks and two died at 4 and 12 weeks as a result of pneumonia.

In the intention-to-treat analysis, 9 (45.0%) of 20 patients in the MMF group, 9 (45.0%) of 20 patients in the tacrolimus group and 6 (30.0%) of 20 patients in the IVC group achieved CR at 24 weeks (P = 0.655). PR occurred in six (30.0%) patients in each group (P = 1.000). When taking both into consideration, 15 (75.0%) patients in the MMF group, 15 (75.0%) patients in the tacrolimus group and 12 (60.0%) patients in the IVC group achieved either complete or PR (P = 0.445; Figure 1).

The baseline urinary protein excretion in the MMF, tacrolimus and IVC groups were 3.66 (0.38–9.87), 3.36 (0.05–7.90) and 3.70 (0.48–6.44) g/24 h, respectively; serum albumin levels were 28 (10–43), 25.5 (10–36) and 23.5 (7–34) g/L, respectively. Proteinuria levels decreased significantly from the baseline after just 4 weeks of therapy in the tacrolimus group (P = 0.0051), whereas they did not change remarkably until 8 weeks in the MMF group and in the IVC group. Serum albumin levels increased significantly from the baseline after 8 weeks of therapy in the tacrolimus group (P = 0.048), whereas they did not change notably until 12 weeks in the other two groups. The urinary protein excretion at 24 weeks in the MMF, tacrolimus and IVC groups were 0.46 (0.04–3.35), 0.28 (0.03–4.42) and 0.39 (0.02–4.93) g/24 h, respectively (P = 0.707); serum albumin levels were 40.2 (26–47), 37.0 (25–45) and 33.2 (14–44) g/L, respectively (P = 0.004) (Figure 2A and B).

Regarding serum creatinine, there were no significant differences among the three groups at baseline (P = 0.2315) and follow-up at 24 weeks (P = 0.0586). One patient withdrew from the study due to a doubling of serum creatinine from a normal level after 8 weeks of tacrolimus treatment; however, it mainly resulted from treatment failure rather than the effects of tacrolimus because no improvement was observed after dosage reduction. In the IVC group, one patient experienced a 30% increase in serum creatinine levels at 24 weeks. By longitudinal analysis at 24 weeks, the serum creatinine levels significantly decreased in the IVC group [87.0 (34–394) versus 66.0 (34–143) μmol/L, P = 0.0129], kept stable in the MMF group [60.5 (39–274) versus 60.5 (46–135) μmol/L, P = 0.5450] and declined with no statistical significance in the tacrolimus group [83.5 (50–423) versus 72 (57–167) μmol/L, P = 0.4736] (Figure 2C).

The number of patients with elevated anti-dsDNA antibody-binding levels and low C3 concentration were similar among the three groups from the baseline to the last follow-up (Table 2).

The mean SLEDAI was 18.5 ± 4.8 at baseline and reduced to 7.7 ± 4.7 at 24 weeks (P < 0.001). Changes in the SLEDAI among the three groups are shown in Figure 3. There was no significant difference in SLEDAI decrease between the three regimens of induction therapy.

**Adverse events**

There were more infections (including severe infections developed in 15 patients requiring in-patient antibiotic therapy) in the IVC group (8/20) and the MMF group (8/20) than the tacrolimus group (3/20) and more hyperglycemia than the tacrolimus group (3/20) and more hyperglycemia in the IVC group (8/20) and the MMF group (8/20). There were more infections (including severe infections) in the IVC group (11/20) and the MMF group (11/20) than in the tacrolimus group (5/20) and more hyperglycemia in the IVC group (8/20) and the MMF group (8/20) than in the tacrolimus group (3/20).

Adverse events in the IVC group included pneumonia at 12 weeks; in the IVC group, one patient withdrew at 4 weeks and two died at 4 and 12 weeks as a result of pneumonia. There were more infections (including severe infections developed in 15 patients requiring in-patient antibiotic therapy) in the IVC group (8/20) and the MMF group (8/20) than the tacrolimus group (3/20) and more hyperglycemia than the tacrolimus group (3/20) and more hyperglycemia in the IVC group (8/20) and the MMF group (8/20) than in the tacrolimus group (3/20), but the results were not statistically significant among three groups (Table 3).

All the deaths were associated with pneumonia (one in the MMF group, one in the tacrolimus group and two in the IVC group, P = 0.765).

**Discussion**

There have been significant improvements concerning the treatment of SLE over the past decades, especially the variety of immunological therapies at different stages. Over the past 10 years, the introduction of MMF has challenged the standard of care in LN, namely the National Institutes of Health regimen of pulsed IVC [2]. A number of controlled prospective studies have shown that MMF may be equivalent to IVC for induction therapy in severe proliferative LN [12–14]. Tacrolimus ointment has been found to be effective for severe refractory cutaneous lupus lesions [11]. Several studies have also shown tacrolimus to be an alternative regimen for induction therapy of LN [15, 16].

In the present study, we found that MMF and tacrolimus as well as IVC showed significant therapeutic response for induction treatment of ALN. The CR and PR rates were similar among the three groups. The CR rates in this study were lower than those described in a randomized study which was as high as 74.2% [5], possibly due to the different definitions of remission between the two studies. Another key point is the duration of the study—Chan assessed remission at 1 year rather than 24 weeks [5]. In addition, there were 20% of patients with concomitant membranous features on biopsy (V + III and V + IV) in this study, while all Chan’s subjects were Class IV nephritis. It is well known that there are more patients with Classes V + III or V + IV being refractory to immunosuppressive therapy than those with Classes IV and III LN [17–19]. The low remission rates across all of the racial/ethnic groups studied in the Aspreva Lupus Management Study highlight the need to investigate the ideal regimen and its duration in patients with LN [8, 20].
Our results were similar to the previous report [21] that tacrolimus possibly results in a faster resolution of proteinuria and hypoalbuminemia in ALN, compared with IVC and MMF therapy. Three patients in the tacrolimus group achieved CR within 4 weeks of starting treatment. Similar findings were reported in adults or children with primary nephrotic syndrome [22, 23]. This is likely due to the antiproteinuric effect of anticalcinurin. The long-term benefit of tacrolimus regarding its ability to rapidly decrease proteinuria needs to be assessed.

The number of patients with elevated anti-dsDNA antibody binding levels and low C3 concentration decreased significantly at 24 weeks from baseline in every treatment group. SLEDAI scores were also markedly lower at 24 weeks than baseline scores in the three groups. These results suggest that both MMF and tacrolimus in combination with prednisone are effective in controlling the systemic activity of SLE.

Adverse events (AEs) were reported at similar rates among the three treatment groups, whereas the most common AE was infection. In total four patients died during the study: one in the MMF group, one in the tacrolimus group and two in the IVC group ($P = 0.765$). All deaths were due to infection and none were due to SLE. Treatment discontinuation owing to AEs was responsible for three study

<table>
<thead>
<tr>
<th></th>
<th>MMF [$n$ (%)]</th>
<th>Tacrolimus [$n$ (%)]</th>
<th>IVC [$n$ (%)]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with elevated anti-dsDNA antibody binding levels at baseline</td>
<td>15 (75.0)</td>
<td>17 (85.0)</td>
<td>15 (75.0)</td>
<td>0.732</td>
</tr>
<tr>
<td>Patients with elevated anti-dsDNA antibody binding levels at 24 weeks</td>
<td>4 (25.0)</td>
<td>4 (23.5)</td>
<td>5 (29.4)</td>
<td>0.881</td>
</tr>
<tr>
<td>Patients with low C3 levels at baseline</td>
<td>16 (80.0)</td>
<td>17 (85.0)</td>
<td>17 (85.0)</td>
<td>0.918</td>
</tr>
<tr>
<td>Patients with low C3 levels at 24 weeks</td>
<td>7 (43.8)</td>
<td>6 (35.3)</td>
<td>6 (35.3)</td>
<td>0.937</td>
</tr>
</tbody>
</table>

Fig. 2. Changes of serum albumin (median, A), urine protein (median, B) and serum creatinine (median, C) from the baseline value at each follow-up evaluation.

Table 2. Changes of immune parameters from the baseline to the last follow-up
withdrawals in the MMF group, two in the tacrolimus group and one in the IVC group. The comparatively high incidence of infection and their corresponding deaths was associated with the following factors: firstly, some patients had long duration of lupus (one patient with 240 months, two with 120 months, two with 96 months, three with 48 months and three with 36 months) were enrolled in this study, who had long-term steroids exposure and easily became the victim of infections; secondly, the severity of the disease should be considered—20% of the enrolled patients had abnormal creatinine concentrations at baseline; thirdly, Chinese patients may not be able to tolerate large dosages of the immunosuppressants, which refer to the previous studies [6, 8]. Moreover, it may have some benefits for reducing the mortality rate when the patients on high-dose GC with/without immunosuppressive agents to be prescribed antibiotics as prophylaxis against pneumonia.

Drug-induced nephrotoxicity is a major concern of the long-term use of the calcineurin inhibitors [24–26]. Although in renal transplant recipients, serum creatinine level after 5 years appears to be lower in patients treated with tacrolimus-based than CsA-based regimens [27], long-term data on renal function in patients with LN treated with tacrolimus are currently lacking. One patient exhibited a doubling of serum creatinine from the normal level after 8 weeks of tacrolimus treatment, it may mainly be caused by treatment failure rather than the adverse effects of tacrolimus because no improvement in renal function was observed after dosage reduction. Although with no statistical significance, a decline tendency of serum creatinine was observed in the tacrolimus group. Therefore, more attention should be paid to potential nephrotoxicity for patients who need to be treated for a long period of tacrolimus.

The incidence of irregular menstruation in the IVC group (20%) seems more than that in the MMF group (5%) and tacrolimus group (0%), although there was no statistical significance. As many lupus patients are young women of child-bearing age, the risk of ovarian toxicity with IVC regime is of major concern [28, 29]. To establish the long-term impact of MMF or tacrolimus on ovarian, a further follow-up is required [30–32].

However, there are several limitations of this study. Firstly, the sample size is relatively small. Larger samples might produce a significant difference among the three groups. Secondly, 24 weeks may be too short to differentiate among the regimes because the disease may continue to improve and AEs may continue to emerge. A longer period of observation on renal flares and renal function preservation is necessary. Finally, the optimal dosage regimen of MMF and tacrolimus remains unclear. Which dose could be more effective but tolerable in Chinese patients has to be explored in future trials.

In summary, this study showed that a 6-month course of MMF or tacrolimus treatment could lead to a significant therapeutic response and may be possible alternatives to IVC as induction therapies for Chinese patients with ALN. Compared with the conventional cytotoxic treatment, tacrolimus possibly results in a faster resolution of proteinuria. Further studies are necessary to determine the optimal dosage and duration of the therapies.

Key messages

- Both MMF and tacrolimus are possible alternatives to IVC as induction therapies for ALN.
- Tacrolimus possibly results in a faster resolution of proteinuria and hypoalbuninemia.

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Conflict of interest statement. None declared.

References
