Low-dose tacrolimus in treating lupus nephritis refractory to cyclophosphamide: a prospective cohort study

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Abstract

Objectives

This study aims to assess the efficacy and safety of low-dose tacrolimus therapy in patients with refractory lupus nephritis (LN) who were resistant to cyclophosphamide (CYC).

Methods

A total of 26 LN patients (4 men and 22 women) with persistent proteinuria who were resistant to CYC treatment (>8 g in less than 6 months) were enrolled. Tacrolimus was initiated at 2 mg/day (if patient weight <60 kg) or 3mg/day (if patient weight ≥60 kg), administered in two divided doses. Prospective data on daily proteinuria, serum albumin level, and serologic lupus activity were collected for 6 months.

Results

Mean age at baseline was 29.36±9.45 years. Mean urinary protein significantly decreased from 6.91±4.50 g at baseline to 1.11±1.10 g at 6 months ($p<0.001$). Mean serum album level significantly increased from 25.56±7.94 g/L at baseline to 38.12±2.42 g/L at 6 months ($p<0.001$). Mean systemic lupus erythematosus disease activity index (SLEDAI) score decreased from 11.42±6.74 at baseline to 3.61±2.73 at 6 months ($p<0.001$). Complete or partial response was observed in 88.46% of patients receiving tacrolimus therapy at 6 months. Twenty-one patients achieved partial or complete remission in two months. There was no significant difference among tacrolimus levels for patients with complete, partial, or no response. The effective dosage in this study was 2–3 mg/day for patients with complete or partial response to tacrolimus. Tacrolimus was well tolerated at the administered dose, though one patient developed severe lung infection.

Conclusion

Our results suggested tacrolimus at low dosage and serum level to be potentially effective and safe for treatment in patients with LN resistant to sufficient CYC therapy. A tacrolimus dosage of 2–3 mg daily appears to be effective and safe.

Key words

systemic lupus erythematosus, lupus nephritis, tacrolimus

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Received on January 27, 2012; accepted in revised form on March 16, 2012.

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Funding: This work was supported by the National Key Technology Research and Development Programme in the 11th Five year Plan of China (no. 2008BAI59B02, no. 2008BAI59B03), and the Chinese SLE Treatment and Research Group.

Competing interests: none declared.

Introduction

Nephritis is a major manifestation of systemic lupus erythematosus (SLE). Up to 60% of patients with SLE develop renal involvement at some stage of their illness (1). Several studies have demonstrated that lupus nephritis (LN) is an important predictor of both renal impairment and global morbidity and mortality in these patients (2, 3). Although corticosteroids rapidly control disease activity, these effects might not be maintained over time (4). A number of trials have demonstrated that patients with LN could be effectively treated with corticosteroids plus immunosuppressive reagents (5, 6); the latter include cyclophosphamide (CYC), cyclosporine, and mycophenolate mofetil (6-8). However, some patients do not respond well to these treatments. Less than 50% of patients achieve complete response to corticosteroid plus CYC treatment, despite significant side effects (9, 10). Those patients who fail to achieve remission tend to have progressive renal deterioration that ultimately leads to end-stage renal disease (2).

To limit CYC-related toxicity and improve efficacy, alternative new therapies have been extensively investigated (7, 8). Tacrolimus is a T-cell-specific calcineurin inhibitor that shares similar immunosuppressive actions with cyclosporine A (CsA). It complexes with immunophilin FK506 binding protein 12, and inhibits the phosphatase activity of calcineurin, resulting in decreased interleukin-2 transcription and inhibition of T-cell activation (11, 12). *In vivo* and *in vitro* studies have shown that tacrolimus activity is 10–100 times more potent than CsA (12). Clinically, tacrolimus was reported to be effective for treatment of active rheumatoid arthritis (13) and LN (14-23). Efficacy and safety of tacrolimus *versus* intravenous CYC as induction therapy was compared in a multi-centre randomised controlled trial. Results indicated that tacrolimus is at least as efficacious as intravenous CYC and has a more favourable safety profile (15). It was reported that tacrolimus at a dose of 0.1 mg/kg/day showed a significant therapeutic response in cases who failed to respond to sufficient intravenous CYC therapy (14). However, the efficacy of low-dose tacrolimus in treating LN resistant to CYC remains largely unknown.

The purpose of this study was to determine the optimal dose and serum level of tacrolimus administration and examine whether low-dose tacrolimus is effective and safe for maintenance treatment in patients with LN resistant to CYC and corticosteroid combination treatment.

Patients and methods

Patient selection

From January 2009 to December 2010, 26 patients with LN resistant to CYC treatment (22 women and 4 men) were enrolled in the study. The inclusion criteria were as follows: (i) fulfilling the revised ACR criteria for SLE; (ii) persistent proteinuria >1.5 g/24 h (with or without active urinary sediments) that was resistant to >8 g i.v. CYC treatment in 6 months, plus prednisone with initial dose ≥ 1.0 mg/kg/day for at least 4 weeks, and CYC treatment halted at least 1 month before the current study; (iii) age range of 16–60 years; (iv) female patients of child-bearing age and male patients agreed to maintain effective birth control practice during the study. Exclusion criteria were: (i) initial serum creatinine ≥200 μmol/L; (ii) pregnancy; (iii) overlapped with other connective tissue disease; (iv) known allergy to tacrolimus or other calcineurin inhibitors; (v) other causes of persistent proteinuria (*e.g.* renal vein thrombosis, infection). Withdrawal criteria were: (i) new onset severe infection; (ii) no effect after 2 months at a dose of 4 mg/day; (iii) serum creatinine increased by more than 30%; (iv) other intolerable adverse events; (v) significant non-compliance with the protocol. Informed consent was obtained from each patient. Patients were treated with a protocol consisting of prednisone and tacrolimus for 1 year. This study was in compliance with the Declaration of Helsinki and was in agreement with the guidelines approved by the ethics committee of Peking Union Medical College Hospital.

Treatment protocol

Corticosteroid: Pre-existing oral prednisone dose was maintained in all pa-

tients at the initiation of oral tacrolimus. The daily dose was gradually decreased by 5 mg per two weeks until 20 mg/day, then by 2.5 mg every 2 weeks until 10 mg/day and maintained throughout the study period.

Tacrolimus: All patients were treated with oral tacrolimus. The initial dose was 2 mg/day (body weight <60 kg) or 3 mg/day (body weight ≥60 kg). If patients did not respond after 2 months of treatment, the dosage was increased to a maximum of 4 mg/day and maintained throughout the study period. Change in tacrolimus dose was generally made in steps of 0.5 mg per 2 weeks. We did not have a target therapeutic tacrolimus level for dosage titration in this study. Tacrolimus serum level in all patients was monitored at 2 weeks, 1 month, 2 months, 4 months and 6 months. After 6 months, tacrolimus was administered at a dose of 1 mg per day for all patients responsive to tacrolimus.

Other medicine: Pre-existing reagents such as angiotensin II receptor antagonists or angiotensin-converting enzyme inhibitors were maintained throughout the study period.

Table I. Baseline characteristics of LN patients treated with tacrolimus.

Characteristics	Number (%), mean ± SD
Sex (male/female)	4/22
Age	29.36 ± 9.45
SLE duration (years)	5.20 ± 5.08
Lupus nephritis duration (years)	2.91 ± 2.74
Extra-renal features	17 (65.38)
Mucocutaneous	8 (30.77)
Musculoskeletal	10 (38.46)
Neuropsychiatric	2 (7.69)
Haematologic	8 (30.77)
Pathologic type	
Class III	5 (19.23)
Class IV	2 (7.69)
Class V	5 (19.23)
Class III+V	7 (26.92)
Class IV+V	4 (15.38)
Unknown	3 (11.54)
Scr (μmol/L)	84.68 ± 37.21
Serum C3 (mg/dl)	0.61 ± 0.21
Proteinuria level	6.91 ± 4.50
1.5–2.9 g/24 h	9 (34.62)
≥3.0g/24h	17 (65.38)
Active urinary casts	19 (73.08)
Serum albumin (g/L)	25.56 ± 7.94
Anti-double-stranded DNA (ELISA)	220.52 ± 275.86
Positivity (Indirect immunofluorescence ≥1:20)	16 (61.54)
White blood cell ($\times 10^9$/L)	4.19 ± 2.02
Hypertension	9 (34.62)
ACEI and/or ARB treatment	10 (38.46)

Table II. The clinical features of patients at the beginning of therapy with CYC.

Characteristics	Number (%), mean ± SD
Age	28.12 ± 10.35
Extra-renal features	20 (76.92)
Mucocutaneous	12 (46.15)
Musculoskeletal	14 (53.85)
Neuropsychiatric	4 (15.38)
Haematologic	10 (38.46)
Scr (μmol/L)	95.67 ± 40.35
Serum C3 (mg/dl)	0.49 ± 0.26
Proteinuria level	7.52 ± 3.61
Active urinary casts	21 (80.77)
Serum albumin (g/L)	27.35 ± 8.26
Anti-double-stranded DNA (ELISA)	360.46 ± 280.52

Treatment evaluation

Patients were evaluated at 2 weeks and monthly for the first 2 months and bi-monthly thereafter. Clinical evaluation included blood pressure and disease activity index (SLEDAI score) testing. Laboratory tests including 24-hour urine tests for proteinuria, as well as blood tests for complete blood counts, serum sugar, serum creatinine, liver enzymes, serum complements, anti-dsDNA antibody and serum tacrolimus. Levels were measured by enzyme-linked immunoabsorbent assay (ELISA) during clinic visits at baseline (the time of starting tacrolimus treatment), and at 1, 2, 4 and 6 months post-initiation. After 6 months, patients had physical examination and laboratory tests including 24-hour urine tests and complete blood counts bi-monthly.

Treatment response

The primary treatment outcome measures were the change in 24-hour urinary protein excretion and serum albumin levels. The primary study end point was complete remission following 6 months of treatment. Secondary end points included complete or partial remission, changes in serum creatinine, serum C3 values, and adverse effects. Complete response and partial response were defined as effective. No response was defined as non-effective. Complete response was defined as urinary protein excretion <0.5 g/day with normal urinary sediment, normal serum albumin concentration (serum albumin ≥35 g/L) and stable kidney function (normal serum creatinine range, or not >15% above baseline values). Partial response was defined as urinary protein excretion in the range of 0.5–2.9 g/24 h and a decrease of at least 50% compared to the baseline level, with a serum albumin concentration of at least 30 g/L, and stabilisation or improvement in serum creatinine (Scr) level. Treatment failure was defined as urinary protein excretion that remained ≥3.0 g/24 h,

decreased <50% of baseline level, or serum albumin concentration <30 g/L.

Statistical analysis

All statistical analyses were performed using SPSS version 16.0 for Windows 7 (IBM, Armonk, NY, USA). Data were expressed as mean ± S.D. Student's *t*-test or ANOVA were used to evaluate the significance of the differences. The effects of treatment group on the serial change in proteinuria, serum album level, and SLEDAI score were compared by linear regression for repeated measures. Statistical significance was defined as a $p<0.05$, using a two-tailed analysis.

Results

Patient characteristics

Table I shows the baseline renal characteristics and extra-renal features of all patients. Table II displays the clinical features of patients at the beginning of therapy with CYC. The mean age was 29.36±9.45 years (range of 17–47 years), the mean duration of SLE was 5.20±5.08 years (range of 1–19 years), and the mean LN duration was 2.91±2.74 (range of 0.5–10 years).

Renal biopsy revealed ISN/RPS class III in five patients, class IV in two patients, class V in five patients, class III+V in seven patients, and class IV+V in four patients. Renal biopsy was not performed in the other three patients, who thus could not be reclassified.

Dosage and serum level of tacrolimus

The initial dose of 2 mg/day (patient weight <60 kg, n=13) or 3 mg/day (patient weight ≥60 kg, n=13) was administered orally in two divided doses. Since daily proteinuria in one patient was elevated at 4 months, the daily tacrolimus dose was increased from 2 mg to 3 mg, resulting in partial response at 6 months. The dose of the other 22 patients who had complete or partial response to tacrolimus was not changed during the 6-month study period. For the three patients who showed no response to tacrolimus with an initial dosage of 3 mg/day (two patients) and 2 mg/day (1 patient), dosage was increased to 4 mg/day after 2 months. However, all three patients remained unresponsive to tacrolimus at 6 months. The effective dosage in this study was 2–3 mg/day for the patients who had complete or partial response to tacrolimus treatment.

A tacrolimus level of <3 ng/mL was effective in all patients with a partial or complete response. The average blood tacrolimus levels of patients with partial or complete response were 1.42±0.50, 1.35±0.79, 1.78±0.89, 1.75±0.53, and 1.75±0.62 ng/mL at 2 weeks, 1 month, 2 months, 4 months, and 6 months respectively. The mean whole blood tacrolimus levels in complete, partial, and no response groups were 1.52±0.36, 2.12±0.63, and 2.46±1.13 ng/mL, respectively. No definite correlation was found between efficacy and tacrolimus level.

Treatment response

One patient was withdrawn from the study at 4 months because of severe pulmonary infection. All other patients completed the study protocol. Following 6 months of tacrolimus therapy, complete and partial response was observed in 23 (88.46%) patients, consisting of ten (38.5%) complete response and 13 (50.0%) partial response patients (Table III, Table IV and Fig. 1). Significant improvement in proteinuria and albumin levels was observed in these patients starting from the first month (Table IV, Fig. 2). Twenty-one patients had achieved partial or complete remission at 2 months (Fig. 1). Tacrolimus treatment had a significant effect on the improvement of proteinuria, serum album level, SLEDAI scores, and complement 3 levels (Table III, Fig. 2). The mean value of proteinuria of patients who achieved partial remission was 1.37±0.80 gram at 6 months.

During the follow-up period of 6 to 12 months, only one patient, who received 1 mg/day tacrolimus treatment, flared up at 12 months with gastrointestinal symptoms and new-onset severe rash, whereas the other patients responsive to tacrolimus maintained good condition without relapse. No patient in this study flared up with significant increase of proteinuria or serum creatinine from 6 to 12 months.

In 9 out of 26 patients who had basal proteinuria <3g/day, 5 patients achieved complete remission and 2 patients partial remission. In the other 17 patients with basal proteinuria ≥3g/day, 5 patients achieved complete remission and 11 patients partial remission (Table V). Although the complete remission rate in basal proteinuria <3g/day was higher than that in proteinuria ≥3g/day, there was no significant difference between them ($p=0.074$), indicating low-dosage tacrolimus is effective in inducing complete remission especially in patients with mild residual proteinuria.

Relationship of outcome to histologic type of lupus nephritis

Patients with LN class V tended to have higher rates of complete or partial response. All the patients with LN class

Table III. Changes in biochemical parameters after treatment from baseline to month 6.

	Baseline	Month 1	Month 2	Month 4	Month 6	F	*p*-value
Daily proteinuria (g)	6.91 ± 4.50	3.36 ± 2.89	1.91 ± 1.36	1.46 ± 1.18	1.11 ± 1.10	48.25	<0.001
Serum albumin (g/L)	25.56 ± 7.94	30.00 ± 6.13	33.83 ± 4.93	36.50 ± 4.27	38.12 ± 2.42	52.74	<0.001
Serum C3 (mg/dL)	0.61 ± 0.22	0.81 ± 0.25	0.86 ± 0.27	0.87 ± 0.23	0.89 ± 0.17	9.61	0.003
SLEDAI scores	11.42 ± 6.74	7.13 ± 2.39	7.13 ± 2.38	6.07 ± 2.22	3.61 ± 2.73	32.72	<0.001
Scr (μmol/L)	84.68 ± 37.21	86.89 ± 37.12	88.74 ± 35.23	83.47 ± 34.70	84.71 ± 34.31	0.41	0.52

Table IV. Patients failing to achieve complete and/or partial remission at month 6.

Patients failing to achieve complete or partial remission at month 6	n (%)
n. of patients achieving partial remission but failing to meet complete remission	13
Proteinuria ≥0.5 g/24 h	12
Serum albumin <3.5 g/dL	3
Active urinary casts	10
n. of patients failing to meet complete or partial remission	3
Proteinuria ≥3.0 g/24 h or decrease <50% from baseline	3
Serum albumin <3.0 g/dL	1

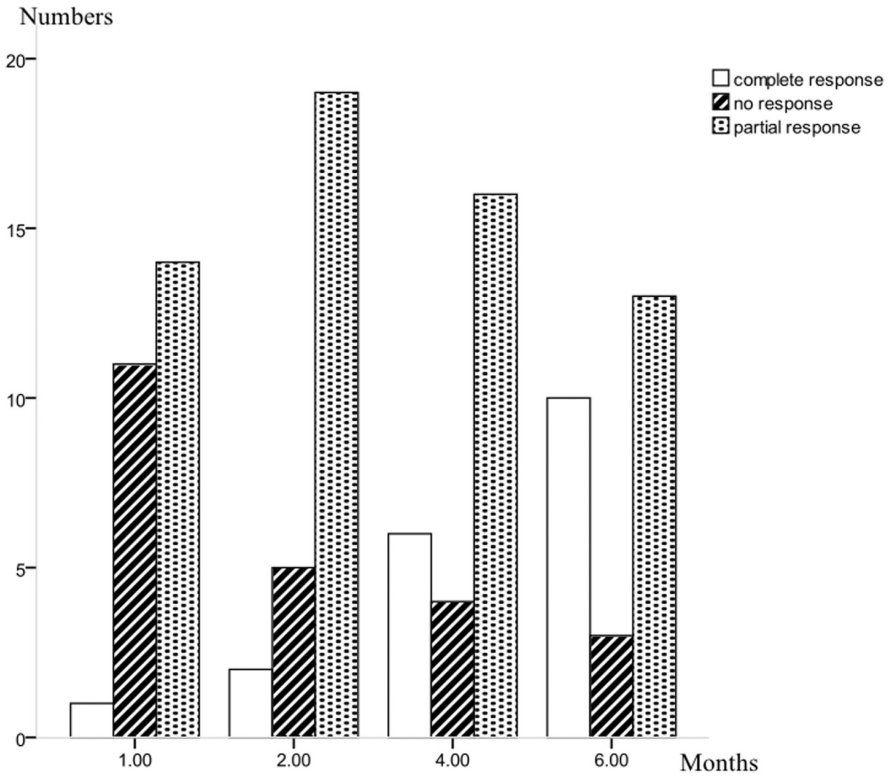

Fig. 1. Number of patients achieving complete remission and partial remission for patients undergoing tacrolimus therapy.

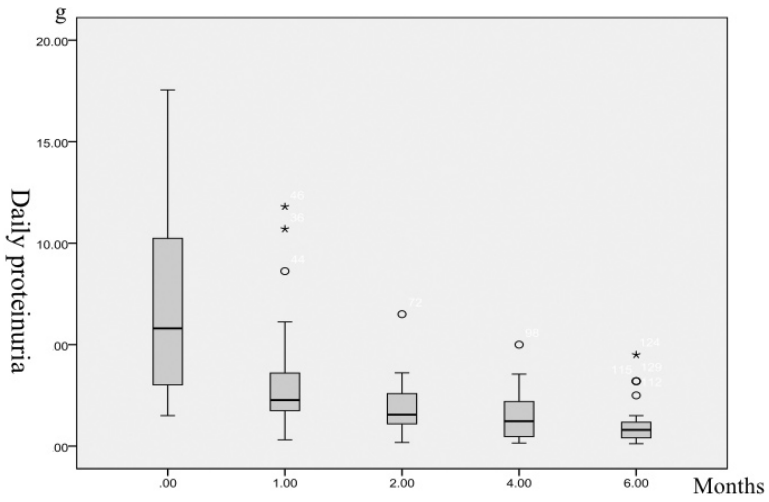

Fig. 2a. Box and whisker plot showing the serial trend of proteinuria during the study period. The boxes indicate median, 25th and 75th percentiles; whisker caps indicate 5th and 95th percentiles; circles and stars indicate outliers.

V, V+IV, or V+III achieved complete or partial remission. Both patients with no response to tacrolimus were classified as histological class III (Table VI).

Adverse events

One patient had severe pulmonary infection at 4 months, infected with aspergillus fumigates and cytomegalovirus. This patient had lupus nephritis for more than 10 years and had used various immunosuppressive drug treatments prior to this study. Only one patient had new onset hypertension and one patient had alopecia. There was no significant increase in the serum creatinine throughout the follow-up period. Other adverse effects did not appear, such as hyperglycemia, liver enzymes elevation and new-onset diabetes.

Discussion

Recent studies have found that tacrolimus is effective in treating active LN (14-23). However, there is little information regarding the role of tacrolimus at low doses in the treatment of lupus nephritis resistant to CYC. We therefore performed a prospective trial in Chinese patients with persistent proteinuria to determine whether a regimen of tacrolimus plus prednisone was partly efficacious for patients resistant to a conventional regimen of cyclophosphamide plus prednisone. The purpose of this clinical study was to explore the efficacy and tolerability of tacrolimus. The population targeted for this study comprised patients who did not sufficiently respond to CYC, as this is the group who would benefit from the therapy. Many patients had been treated with >8 g for less than 6 months of CYC and had taken corticosteroids, yet still had persistent proteinuria. Although six months might be early, as the time required to achieve disease remission can be longer, it is not ethical to continue previous treatment for the patients that were not responsive to it for 6 months. In clinical practice, we found it was hard for most of non responders to achieve additional remission if we continued the previous treatment regimen without change after 6 months. Also the patients compliance will be poor if the treatment regimen is not effective for 6 months. This is why we would like to find if tacrolimus is effective in these LN that failed CYC >8g in 6 months.

We found that a 6-month course of tacrolimus at a dose of 2–3 mg daily was a safe and effective treatment of LN resistant to CYC. Tacrolimus was effective in 23 out of the 26 trial patients. Ten patients have achieved complete remission. Significant improvement in proteinuria and albumin levels was observed in most patients, beginning in month one of the study period. These results suggested that tacrolimus therapy was associated with a rapid improvement of daily proteinuria and serum album level in treating LN resistant to CYC treatment. The patients with basal proteinuria <3g/day had a higher complete remission rate than those ≥3g/day, which told us low-dos-

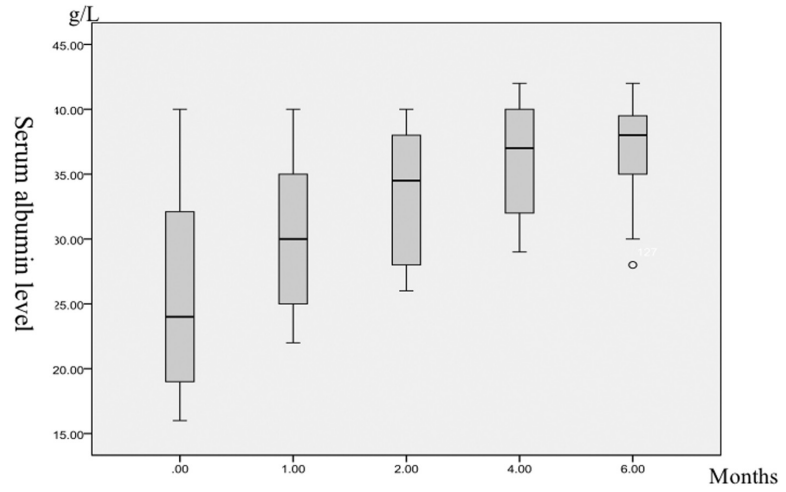

Fig. 2b. Box and whisker plot showing the serial trend of serum albumin level during the study period. The boxes indicate median, 25th and 75th percentiles; whisker caps indicate 5th and 95th percentiles; circles indicate outliers.

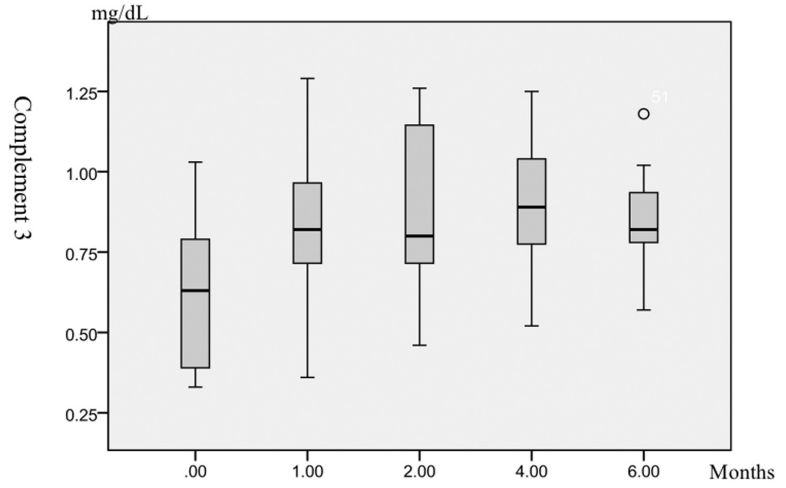

Fig. 2c. Box and whisker plot showing the serial trend of complement 3 level during the study period. The boxes indicate median, 25th and 75th percentiles; whisker caps indicate 5th and 95th percentiles; circles indicate outliers.

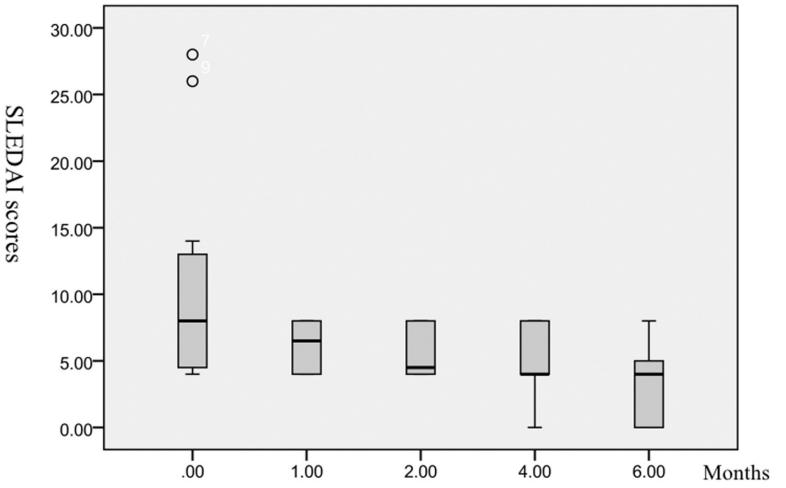

Fig. 2d. Box and whisker plot showing the serial trend of SLEDAI scores during the study period. The boxes indicate median, 25th and 75th percentiles; whisker caps indicate 5th and 95th percentiles; circles indicate outliers.

age tacrolimus was effective in inducing complete remission especially in patients with mild residual proteinuria. Previous studies suggested that the optimal serum level of tacrolimus was 3–8 ng/mL or 5–10 ng/mL (15, 18). A daily dose of 0.1–0.2 mg/kg/day was administered in two divided doses (18). This dosage of tacrolimus was lower than previous studies in LN patients. We determined that a serum tacrolimus level of <3 ng/mL was effective in patients with persistent proteinuria. Although the dosage of tacrolimus was increased to 4 mg/day, the three patients who had no response to 2–3 mg/day initially remained non-responsive to tacrolimus at a higher dosage. Therefore, we hypothesised that a tacrolimus dose between 2 and 3 mg daily might be optimal for treating this population of LN patients. Although this effect at early stage might be partially due to the effect of steroid or the delayed effect of CYC treatment, as a proportion of these LN patients achieved partial remission or even complete remission in as early as one month after the treatment was switched from CYC to tacrolimus, we believed tacrolimus was effective in treating LN in these patients that were not responsive to CYC, and the effectiveness was not due to glucocorticosteroid, as all the patients in this study had no response for high-dose prednisone therapy for at least 6 months and the dosage of glucocorticosteroid was gradually tapered after the initiating of tacrolimus.

In this study, patients with LN class V tended to have higher rates of remission. All the patients with LN class V, V+IV, or V+III achieved complete or partial response. Both patients with no response to tacrolimus were classified as histologic class III.

In general, tacrolimus therapy at the dose range described in this study was well tolerated, and side-effects were minor, except for one patient who developed severe lung infection. At the described dosage, tacrolimus was not associated with hyperglycemia or significant increase in serum creatinine levels. Unlike CYC, prolonged amenorrhea or haemorrhagic cystitis was not observed. This lower incidence of

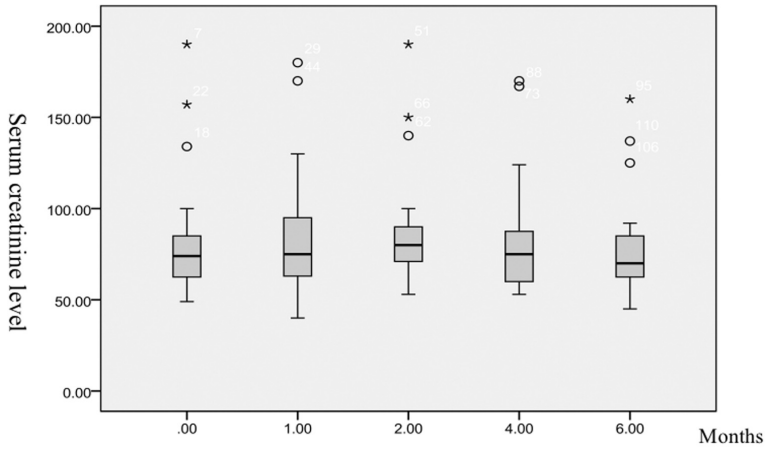

Fig. 2e. Box and whisker plot showing the serial trend of Scr during the study period. The boxes indicate median, 25th and 75th percentiles; whisker caps indicate 5th and 95th percentiles; circles and stars indicate outliers.

Table V. Association between complete or partial remission with basal value of proteinuria.

	Basal proteinuria ≥3g/day	Basal proteinuria <3g/day	Total
Complete response	5	5	10
Partial response	11	2	13
No response	1	2	3
Total	17	9	26

Table VI. Association of kidney pathologic type with treatment response.

Pathologic type	Complete response	Partial response	No response
Class III	2	1	2
Class V	4	1	0
Class IV	1	1	0
Class III+V	2	5	0
Class IV+V	1	3	0
Unknown	0	2	1

side-effects is the major advantage of tacrolimus use in SLE patients.

In summary, we found that a 6-month course of tacrolimus at a dosage of 2–3 mg daily is a safe and effective treatment of LN with persistent proteinuria resistant to CYC, which was never reported previously.

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