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ORIGINAL ARTICLE

Efficacy and safety of tacrolimus for lupus nephritis: a placebo-controlled double-blind multicenter study

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Abstract We evaluated the efficacy and safety of tacrolimus in patients receiving glucocorticoid therapy for lupus nephritis. Patients with persistent nephritis were randomized to receive 28 weeks of double-blind treatment with tacrolimus (3 mg/day) or placebo. The primary endpoint was the change in the lupus nephritis disease activity index (LNDAI) calculated from scores for daily urinary protein excretion, urinary red cells, serum creatinine, anti-double-stranded DNA antibody, and serum complement. Statistical analysis was performed using the full analysis set. The LNDAI was decreased by 32.9 ± 31.0% (mean ± SD) in the tacrolimus group ($n = 28$) and was increased by 2.3 ± 38.2% in the placebo group ($n = 35$) at final evaluation. There was significant improvement in the tacrolimus group. Daily urinary protein excretion showed a significant decrease in the tacrolimus group ($p < 0.001$). The complement (C3) level showed a significant increase in the tacrolimus group ($p = 0.001$). Treatment-related adverse events occurred in 92.9% of the tacrolimus group and 80.0% of the placebo group, but the difference was not significant. In patients on glucocorticoid therapy for lupus nephritis, addition of tacrolimus to basal therapy achieved significant improvement compared with placebo. Tacrolimus may therefore be a useful alternative treatment for lupus nephritis.

Keywords Immunosuppressive drugs · Lupus nephritis · Randomized controlled trial · Systemic lupus erythematosus · Tacrolimus

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Introduction

Lupus nephritis is the nephropathy associated with systemic lupus erythematosus (SLE). It can progress to end-stage renal disease that requires hemodialysis or transplantation, and may even be fatal. The etiology of lupus nephritis is not fully understood. Because an increase in anti-DNA antibodies and a decrease in serum complement is detected in these patients, the production of autoantibodies by abnormally activated immune cells, the deposition of immune complexes in the glomeruli, and a consequent inflammatory reaction are likely to be involved.

The treatment of lupus nephritis mainly involves remission induction therapy in the acute stage and maintenance therapy thereafter [1]. Acute remission induction therapy includes treatment with high doses of steroids for intensive immunosuppression, initial cyclophosphamide pulse therapy, or combined therapy with a steroid and an immunosuppressant. During maintenance therapy after remission has been achieved, the steroid is tapered and its maintenance dose is determined, while combined therapy with an immunosuppressant is considered depending on the changes in laboratory parameters such as urinary protein excretion, C3, anti-DNA antibody, and serum creatinine.

Steroids are frequently used to achieve remission in the acute stage, and it is possible to obtain remission with

steroid therapy alone in many patients. However, complete remission is not achieved in some patients using this therapy, and adequate control is not obtained even though disease activity may not be high. In patients who relapse after remission has been achieved, the steroid dose is increased, leading to concern over the adverse effects of long-term steroid therapy (such as osteoporosis, necrosis of the femoral head, cataract, etc.) that can markedly influence daily activities. In some patients, remission cannot be induced by steroid therapy alone because adverse reactions prevent the continuation of therapy or the use of sufficiently high doses, or else steroids cannot be tapered due to persistent disease activity. When the disease is poorly controlled for a long period, dialysis or renal transplantation may sometimes become necessary and this influences the prognosis. In these circumstances, immunosuppressants can also be used to achieve better control of disease activity [2].

Various immunosuppressants have been employed for the treatment of lupus nephritis, including cyclophosphamide, azathioprine, mycophenolate mofetil, and cyclosporine. The investigator's group of the National Institutes of Health reported that pulse therapy with cyclophosphamide improves the prognosis of lupus nephritis compared with glucocorticoid therapy alone [3], and cyclophosphamide pulse therapy is now often used to treat glucocorticoid-refractory proliferative lupus nephritis. However, cyclophosphamide is an alkylating agent that can cause malignant tumors and early menopause due to ovarian atrophy, and is strongly teratogenic [4]. It was reported [5] that azathioprine is more effective and safe as a maintenance therapy for proliferative lupus nephritis than long-term cyclophosphamide pulse therapy. However, another study [6] suggested that azathioprine was only slightly more effective than placebo and should not be used alone. Mycophenolate mofetil is an inhibitor of purine metabolism that was developed to control rejection after organ transplantation, and its efficacy for lupus nephritis has recently been reported [7, 8]. Cyclosporine is an immunosuppressant that inhibits the production of interleukin-2 (IL-2), a T cell-activating factor. Recent reports [9, 10] have suggested that it is effective for severe SLE and lupus nephritis, but other studies [11, 12] indicated that cyclosporine does not improve the serum complement level and does not reduce disease activity.

Tacrolimus is another immunosuppressant that blocks T cell activation by specifically inhibiting calcineurin. It is used to control rejection after kidney, liver, heart, and bone marrow transplantation, and is also used to control graft-versus-host disease. In Japan, tacrolimus is additionally employed to treat myasthenia gravis [13, 14], rheumatoid arthritis [15], and atopic dermatitis (as a topical preparation) [16]. Regarding the efficacy of tacrolimus for lupus nephritis, it has been shown to prolong survival and decrease proteinuria in murine models of SLE (MRL/lpr mice and NZB/NZWF1 mice) [17]. In humans, its efficacy has been suggested by a few case reports [18, 19], but there have been no randomized double-blind clinical trials of this agent. Therefore, we conducted a placebo-controlled double-blind clinical study to assess the efficacy and safety of tacrolimus therapy for inducing remission in patients with active nephritis and immune dysfunction in whom disease activity was not very high but was persistent, making it difficult to maintain the steroid dose at 10 mg/day or lower.

Patients and methods

Patients

This trial was conducted at 29 study centers from July 2003 to May 2005.

Patients were eligible if lupus nephritis was diagnosed according to the 1982 revised criteria for the classification of SLE from the American College of Rheumatology [20]. Other enrollment criteria were treatment with glucocorticoids at a daily dose $\geq$10 mg (as prednisolone equivalent) and difficulty in tapering therapy. Eligible patients had been on glucocorticoid therapy at a daily dose $\geq$10 mg for at least eight weeks prior to administration of the study drug, and the attending physician had judged that it was difficult to reduce the dose to below 10 mg/day because of the possibility of recurrence. Eligible patients also had clinical evidence of persistent nephritis with serologic abnormalities (proteinuria $\geq$0.5 g/day and/or urinary red blood cell (RBC) count $\geq$21/hpf; anti-double-stranded (ds)-DNA antibody >10 IU/mL (normal is less than 10 IU/mL) and/or serum complement (C3) <84 mg/dL (normal: 84–151 mg/dL)). The lupus nephritis disease activity index (LNDAI), which is a composite score that is described below, and was the primary endpoint of this study, was required to be 3 or more for enrollment. The eligible age range was $\geq$16 to <65 years.

Patients were excluded if they had started glucocorticoid treatment or increased the glucocorticoid dosage within eight weeks of the start of the trial. Patients were also excluded if they had started or increased the dosage of any concomitant medication that could influence proteinuria within eight weeks of the start of the trial (antiplatelet agents, anticoagulants, urokinase, nonsteroidal anti-inflammatory drugs, calcium antagonists, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and Chinese herbal medicines). Furthermore, patients were excluded if they had received glucocorticoid pulse therapy or plasmapheresis (plasma exchange or immunoabsorption) within 12 weeks of the start of the trial.

Finally, patients were excluded if they had started or increased the dosage of another immunosuppressant within 12 weeks of the start of the trial, but patients were not excluded if they had received a fixed dosage of another immunosuppressant during that period. (Immunosuppressant therapy more than 12 weeks before enrollment was not specified.) Patients were excluded if they had received cyclophosphamide pulse therapy within 24 weeks before the start of the trial. Other exclusion criteria were a serum creatinine level of $\geq$1.5 mg/dL, pancreatitis, abnormal glucose tolerance (fasting blood glucose $\geq$110 mg/dL, postprandial glucose $\geq$200 mg/dL, or hemoglobin (Hb) A_{1c} $\geq$5.9%), abnormal liver function (AST, aspartate aminotransferase or ALT, alanine aminotransferase) $\geq$2.5 times the upper limit of normal), women who were pregnant, breastfeeding, or who wished to become pregnant during the study period, and patients who were unsuitable for other reasons according to the judgment of the attending physician.

Methods

This was a randomized multicenter placebo-controlled double-blind study. Sixty-three patients were assigned randomly to the tacrolimus group or the placebo group and were treated with oral tacrolimus (3 mg/day) or placebo once daily after dinner for 28 weeks. After starting the trial, the glucocorticoid dose could not be increased, although tapering was allowed. Concomitant use of the following medications was not permitted: other immunosuppressants (mizoribine, cyclophosphamide, azathioprine, cyclosporine, mycophenolate mofetil, etc., including other tacrolimus preparations), potassium-sparing diuretics, live vaccines, and bosentan hydrate. The following were also not allowed: glucocorticoid pulse therapy, plasma exchange, hemodialysis, and surgical procedures requiring hospitalization. Changing the dose or starting any of the following medications was not allowed during the study period because it could influence proteinuria: antiplatelet agents (dipyridamole and dilazep), anticoagulants (heparin and warfarin), urokinase, nonsteroidal anti-inflammatory drugs (except for topical agents), calcium antagonists, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and Chinese herbal medicines. However, small dose changes and short-term use were allowed if complications worsened or adverse events developed.

The present trial was carried out in accordance with the Declaration of Helsinki. This trial was conducted according to a protocol prepared in accordance with the Japanese GCP standards, which conform with ICH-GCP. All participating institutions received the approval of their governing institutional review board or equivalent, and all patients provided voluntary written informed consent.

Study endpoints

The severities of five parameters (daily urinary protein excretion, urinary RBC count, serum creatinine, anti-ds-DNA antibody, and the complement (C3) level) were scored and the LNDAI was calculated as the total of these scores. The primary endpoint was the change in this index. The parameters were scored as follows: daily urinary protein excretion scored 0 for <0.3 g/day, 1 for 0.3–0.99 g/day, 2 for 1.0–3.49 g/day, and 3 for $\geq$3.5 g/day; urinary RBC count scored 0 for $\leq$5/hpf, 1 for 6–20/hpf, 2 for 21–50/hpf, and 3 for $\geq$51/hpf; serum creatinine scored 0 for $\leq$1.0 mg/dL, 1 for 1.01–1.3 mg/dL, 2 for 1.31–1.8 mg/dL, and 3 for >1.8 mg/dL in men, while the scores were 0 for $\leq$0.8 mg/dL, 1 for 0.81–1.1 mg/dL, 2 for 1.11–1.6 mg/dL, and 3 for >1.6 mg/dL in women; anti-ds-DNA antibody scored 0 for $\leq$10 IU/mL, 1 for 11–30 IU/mL, 2 for 31–50 IU/mL, and 3 for >50 IU/mL; and complement (C3) scored 0 for $\leq$84 mg/dL, 1 for 72–83.9 mg/dL, 2 for 60–71.9 mg/dL, and 3 for <60 mg/dL.

The total scores at the initial and final evaluations (or at the time of discontinuation) were calculated and the change in the total score was assessed. Then the mean change was compared between the two groups. The secondary endpoints were daily urinary protein excretion, urinary RBC count, serum creatinine, anti-ds-DNA antibody, complement C3, systemic lupus erythematosus disease activity index (SLEDAI) [21], creatinine clearance, and glucocorticoid dose. The percentage of patients in whom daily urinary protein excretion decreased to less than 0.3 g/day was calculated, as well as the normalization rates for urinary RBC, anti-dsDNA antibody, and the complement (C3) level. The percentage of patients whose serum creatinine level remained within the standard range was also calculated. To assess safety, we evaluated adverse events and laboratory abnormalities. sCr, anti-ds-DNA antibody, and C3 were measured at the central laboratory, but daily urinary protein excretion and the urinary RBC count were not. We also obtained blood samples at 12 ± 4 h after the administration of tacrolimus and measured serum concentrations.

Statistical analysis

Primary analyses were performed on the full analysis set. Significance was set at $p < 0.05$ (two-tailed) and 95% confidence intervals were calculated. Imbalances between the groups were evaluated at $p < 0.15$ (two-tailed) to confirm the homogeneity of background factors. Statistical analysis for imbalances was performed with Fisher's exact test, the t test, and the Mann–Whitney U test, as appropriate. When an imbalance was found between the groups ($p < 0.15$), analysis of covariance was used to adjust the

imbalance and to evaluate whether it influenced the primary endpoint. Otherwise, primary analysis was done without adjustment. The primary endpoint for assessing efficacy was the change in the LNDAI at final assessment (compared between the two groups by the *t* test). The secondary endpoints were the parameters of disease activity, SLEDAI, creatinine clearance, and the glucocorticoid dose, which were compared between the two groups by the Mann–Whitney *U* test. The paired *t* test and Wilcoxon's signed rank test were used for comparison between the baseline and each time point within each group. The rates of adverse events and treatment-related adverse events were calculated and compared between the groups by Fisher's exact test.

This trial is registered at ClinicalTrials.gov (NCT00429377).

Results

Subject disposition

This trial was conducted at 29 institutions, with 2.2 ± 1.6 patients (mean ± SD) per institution, ranging from one to nine per institution. Although renal biopsy was not done at the start of the trial, the results obtained previously are listed for reference. The profiles of the two groups are shown in Table 1. There were no significant differences in background factors between the groups. Both anti-DNA antibody and complement (C4) showed differences between the groups ($p < 0.15$). Anti-DNA antibody was lower in the tacrolimus group than in the placebo group ($p = 0.146$), while complement (C4) was higher in the tacrolimus group ($p = 0.136$). For these two parameters,

Table 1 Profile of the subjects

	Tacrolimus ($n = 28$)	Placebo ($n = 35$)	p value
Gender [no., female (%)]	23 (82.1)	29 (82.9%)	1.000[a]
Age [mean (SD), years]	37.5 (10.8)	35.5 (9.2)	0.435[b]
Duration of lupus nephritis [mean (SD), years]	7.8 (5.1)	7.9 (4.5)	0.898[b]
Duration of confirmed SLE [mean (SD), years]	9.5 (5.3)	9.2 (4.9)	0.828[b]
Renal biopsy according to WHO classification [no. (%)]			
Normal (Class I)			
Mesangial (Class II)		2 (5.7)	
Focal segmental (Class III)	4 (14.3)	6 (17.1)	
Diffuse proliferative (Class IV)	7 (25.0)	12 (34.3)	
Membranous (Class V)	8 (28.6)	9 (25.7)	
Sclerosing (Class VI)			
Normal (Class I) + mesangial (Class II)		1 (2.9)	
Mesangial (Class II) + focal segmental (Class III)		1 (2.9)	
Unavailable	9 (32.1)	4 (11.4)	
Lupus nephritis disease activity index (mean [SD])	5.3 (2.1)	5.2 (1.7)	0.861[b]
Daily urinary protein [median (IQR), g/day]	1.64 (1.21–2.93)	1.47 (0.87–2.91)	0.347[c]
Urinary RBC count [median (IQR), count/hpf]	4.0 (2.0–19.0)	4.0 (2.0–9.0)	0.516[c]
Serum creatinine [median (IQR), mg/dL]	0.67 (0.59–0.87)	0.71 (0.58–0.79)	0.967[c]
Anti-ds-DNA antibody [median (IQR), U/mL]	10.5 (5.0–26.5)	13.0 (5.0–24.0)	0.950[c]
Anti-DNA antibody [median (IQR), IU/mL]	16.5 (5.5–50.0)	40.0 (8.9–70.0)	0.146[c]
Complement (C3) [median (IQR), mg/dL]	72.5 (58.0–81.5)	70.0 (58.0–80.0)	0.653[c]
Complement (C4) [median (IQR), mg/dL]	11.0 (8.5–15.0)	8.0 (6.0–14.0)	0.136[c]
Complement (CH50) [median (IQR), U/mL]	20.5 (14.8–30.0)	18.0 (13.2–26.1)	0.341[c]
SLEDAI [median (IQR)]	10.0 (8.0–12.5)	10.0 (8.0–16.0)	0.994[c]
Creatinine clearance [median (IQR), mL/min]	101.4 (66.7–117.0)	95.8 (74.6–121.0)	0.934[c]
Glucocorticoid dose [median (IQR), mg/day]	14.0 (10.0–16.9)	12.5 (10.0–15.0)	0.511[c]

Data are the mean (SD), number of patients (%), or median (IQR)

[a] Fisher's exact test

[b] *t* test

[c] Mann–Whitney *U* test

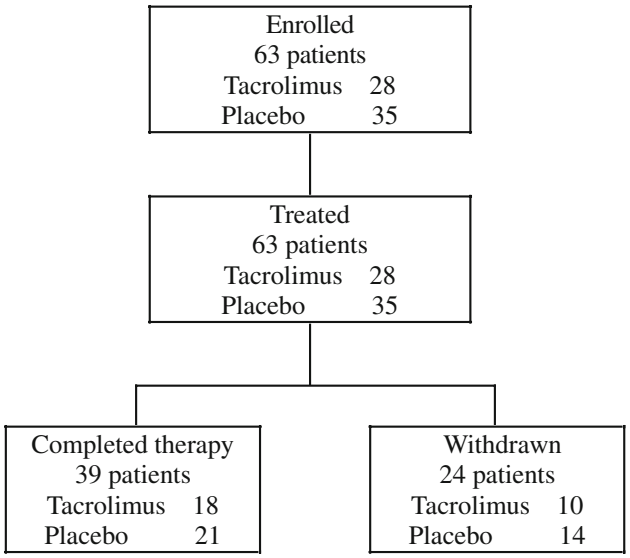

Fig. 1 Subject disposition

adjustment was done by analysis of covariance. As a result, it was found that the imbalances did not influence the primary endpoint and that the two groups were comparable. The five parameters of the LNDAI showed no significant differences between the two groups.

A flow chart showing the dispositions of the subjects is displayed in Fig. 1. Sixty-three patients were enrolled in this trial (28 in the tacrolimus group and 35 in the placebo group), and all 63 patients were treated with the assigned medication. Thirty-nine patients completed the 28-week treatment period (18 in the tacrolimus group and 21 in the placebo group), while 24 patients discontinued treatment (10 and 14, respectively). More patients from the placebo group than the tacrolimus group discontinued treatment because of lack of efficacy (ten patients versus two patients). One patient from the placebo group discontinued due to withdrawal of consent. Regarding the use of restricted concomitant drugs during the trial period, there were no changes in dosage. However, nine patients from the tacrolimus group and eight patients from the placebo group used nonsteroidal anti-inflammatory drugs for the common cold or menstrual pain. Although analysis was performed on the full analysis set by the ITT method, one patient each from the tacrolimus group and the placebo group were excluded from analysis because of no data on the primary endpoint (LNDAI).

Endpoints

The LNDAI decreased by 32.9 ± 31.0% (mean ± SD) in the tacrolimus group and increased by 2.3 ± 38.2% in the placebo group. There was significant improvement of this index in the tacrolimus group (Table 2) ($p < 0.001$).

Table 2 Changes in the lupus nephritis activity index

LNDAI	Tacrolimus ($n = 27$) mean ± SD	Placebo ($n = 34$) mean ± SD
% Change	−32.9 ± 31.0**	2.3 ± 38.2
Absolute change	−1.8 ± 1.9	0.0 ± 2.0

** $p < 0.001$ compared with placebo, t test

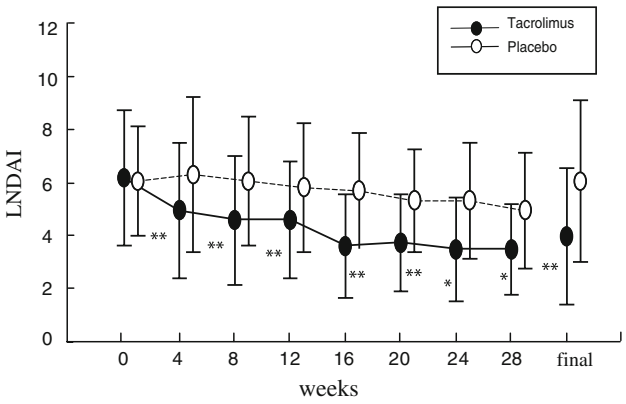

Fig. 2 Changes in the lupus nephritis disease activity index (LNDAI). *$p < 0.05$, **$p < 0.01$, t test, statistical analysis: difference between baseline and each time point compared with placebo

Figure 2 shows the change in LNDAI. There were significant differences between the two groups with respect to LNDAI at each assessment time from week 4 of administration to the final evaluation ($p < 0.001$). In the placebo group, there was no significant difference between the pretreatment and posttreatment values (5.2 ± 1.7 vs. 5.2 ± 2.5), while the tacrolimus group showed a significant decrease in the posttreatment value compared with the pretreatment value (from 5.3 ± 2.1 to 3.5 ± 2.1, $p < 0.001$). Figure 3 shows the changes in daily urinary protein excretion and complement (C3). There were significant differences between the two groups with respect to daily urinary protein excretion and the complement (C3) level at almost every assessment time from week 4 of administration to the final evaluation ($p < 0.001$, $p = 0.001$). In addition, no significant changes in blood pressure were observed in both groups. With respect to urinary RBC, the baseline value (median) was 4.0/hpf and the final value was 4.0/hpf in the tacrolimus group versus 4.0/hpf and 4.0/hpf in the placebo group. For serum creatinine, the respective values were 0.67 and 0.72 mg/dL in the tacrolimus group versus 0.71 and 0.69 mg/dL in the placebo group. For anti-ds-DNA antibody, the baseline and final levels were, respectively, 10.5 and 8.0 U/mL in the tacrolimus group versus 13.0 and 9.5 U/mL in the placebo group. There were no significant differences in these parameters between the two groups at the final evaluation.

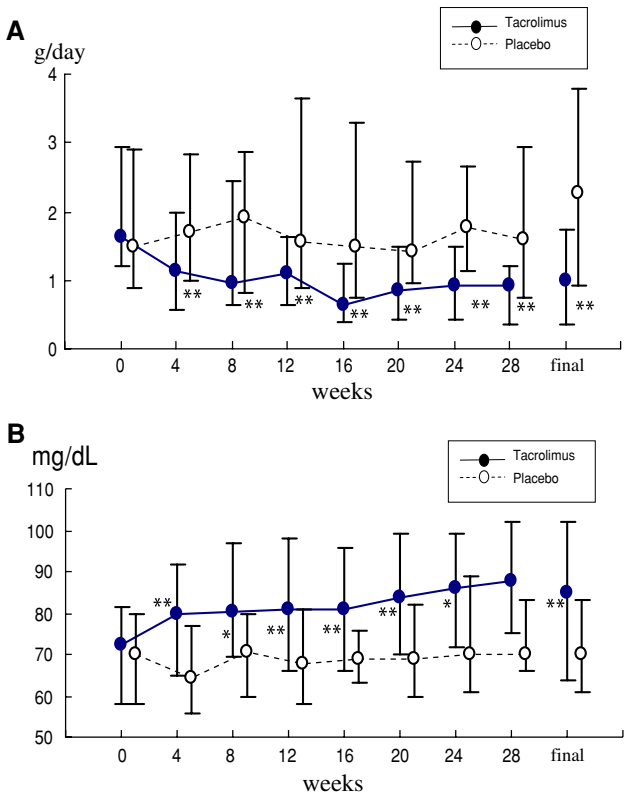

Fig. 3 Changes in the component of the lupus nephritis disease activity index. **a** Daily urinary protein. **b** Complement (C3). Median (IQR), $*p < 0.05$, $**p < 0.01$, Mann–Whitney U test, statistical analysis: difference between baseline and each time point compared with placebo

The percentage of patients in whom daily urinary protein excretion decreased to less than 0.3 g/day was 14.8% (4/27 patients) in the tacrolimus group and 3.0% (1/33 patients) in the placebo group. The normalization rates for urinary red blood cells, anti-ds-DNA antibody, and complement (C3) in the tacrolimus group and the placebo group were, respectively, 33.3% (4/12 patients), 50.0% (7/14 patients), 38.1% (8/21 patients), 33.3% (5/15 patients), 21.1% (4/19 patients), and 21.2% (7/33 patients). Serum creatinine remained within the standard range in 91.7% (22/24 patients) of the tacrolimus group and 89.7% (26/29 patients) of the placebo group (Table 3). There were no significant differences in these items between the two groups.

For serum albumin, the baseline and final levels were, respectively, 3.5 ± 0.5 mg/dL (mean ± SD) and 3.7 ± 0.5 mg/dL in the tacrolimus group versus 3.4 ± 0.6 and 3.3 ± 0.7 mg/dL in the placebo group, with no significant differences between the two groups.

The median total SLEDAI score was 10.0 at baseline and 8.0 at final evaluation in the tacrolimus group versus 10.0 and 9.0 in the placebo group. There were no significant differences between the initial and final scores, but the change in the total score showed a significant difference between the groups in week 28 (33.3% decrease in the tacrolimus group versus 0.0% change in the placebo group, $p = 0.026$).

Table 3 Normalization rates for LNDAI items

Item	Tacrolimus	Placebo
Daily urinary protein excretion[a]	14.8 ($n = 27$)	3.0 ($n = 33$)
Urinary RBC count[b]	33.3 ($n = 12$)	33.3 ($n = 15$)
Anti-ds-DNA antibody[c]	50.0 ($n = 14$)	21.1 ($n = 19$)
Complement (C3)[d]	38.1 ($n = 21$)	21.2 ($n = 33$)
Maintenance of normal serum creatinine	91.7 ($n = 24$)	89.7 ($n = 29$)

Data are presented as percentages

[a] Percentage of patients with <0.3 g/day at final evaluation

[b] Percentage of patients with <5 cells/hpf at final evaluation

[c] Percentage of patients with ≤10 IU/mL at final evaluation

[d] Percentage of patients with ≥84 mg/dL at final evaluation

The median glucocorticoid dose was 14.0 mg/day at baseline and 13.9 mg/day at final evaluation in the tacrolimus group versus 12.5 and 12.0 mg/day in the placebo group, showing little change in either group. There was no significant difference between the two groups with regard to the cumulative glucocorticoid dose during the trial period.

The baseline median creatinine clearance was 95.8 mL/min in the placebo group and 101.4 mL/min in the tacrolimus group, while it decreased to 93.4 and 79.1 mL/min, respectively, in week 12 ($p = 0.005$). At the final evaluation, however, creatinine clearance was 92.9 mL/min in the placebo group and 78.2 mL/min in the tacrolimus group, showing no significant difference ($p = 0.060$). When we calculated eGFR using the GFR in the Japanese equation [22], the baseline median eGFR was 76.5 mL/min/1.73 m^2 in the placebo group and 77.0 mL/min/1.73 m^2 in the tacrolimus group, while it decreased to 75.2 and 77.4 mL/min/1.73 m^2, respectively, in week 12 ($p = 0.056$). At the final evaluation, however, eGFR was 77.3 mL/min/1.73 m^2 in the placebo group and 74.9 mL/min/1.73 m^2 in the tacrolimus group, showing no significant difference ($p = 0.857$).

Adverse events

All 63 patients were evaluated for safety (28 in the tacrolimus group and 35 in the placebo group). Among all the adverse events that occurred, treatment-related events are shown in Table 4. The difference in incidence between the groups was not significant ($p = 0.277$, Fisher's exact test). The incidence of renal dysfunction was high in both groups, while the incidence of gastrointestinal events and

Table 4 Adverse events

	Tacrolimus ($n = 28$) 26 (92.9%)	Placebo ($n = 35$) 28 (80.0%)
Symptomatic events		
Cardiovascular		
Acute myocardial infarction	2 (7.1%)	
Hypertension	2 (7.1%)	3 (8.6%)
Gastrointestinal		
Nausea	4 (14.3%)[a]	
Stomatitis		2 (5.7%)
Nervous system		
Headache		3 (8.6%)
Migraine	2 (7.1%)	
Weight gain		2 (5.7%)
Laboratory data		
Renal dysfunction		
Blood creatinine increased	2 (7.1%)	4 (11.4%)
Creatinine clearance decreased	2 (7.1%)	
Blood uric acid increased		3 (8.6%)
Urine β_2 microglobulin increased	3 (10.7%)	6 (17.1%)
NAG increased	7 (25.0%)	6 (17.1%)
Glucose intolerance		
Blood glucose increased	4 (14.3%)[a]	
Glycosylated hemoglobin increased	2 (7.1%)	
Urine glucose positive	3 (10.7%)	
Others		
Hemoglobin decreased		2 (5.7%)
White blood cell count increased	2 (7.1%)	
AST increased	2 (7.1%)	
Blood LDH increased	2 (7.1%)	
γ-GTP increased		2 (5.7%)
Blood albumin decreased		2 (5.7%)
Blood urea increased	2 (7.1%)	2 (5.7%)
β_2 microglobulin increased		4 (11.4%)
Blood amylase increased		3 (8.6%)
Blood cholesterol increased	2 (7.1%)	3 (8.6%)
Blood triglycerides increased		2 (5.7%)
Infections		
All	16 (57.1%)	20 (57.1%)
Serious	2 (7.1%)	1 (2.9%)

Only treatment-related events that occurred in ≥5% of patients from either group are shown, except for infections. All infections are shown irrespective of the relation to treatment. Data indicate the number of events (%)

[a] $p < 0.05$ compared with placebo (Fisher's exact test)

glucose intolerance tended to be higher in the tacrolimus group. With regard to the blood sugar level, there was a significant difference between the tacrolimus and placebo groups ($p = 0.034$). Tremor, a well-known neurological effect of tacrolimus, did not occur in either group. Adverse events that were not related to treatment occurred in 27 patients (96.4%) from the tacrolimus group and 34 patients (97.1%) from the placebo group, with no significant difference between the two groups ($p = 1.000$, Fisher's exact test).

There was one sudden death in the placebo group, which was suspected of being due to acute heart failure. Four serious adverse events occurred in four patients from the tacrolimus group (two cases of acute myocardial infarction, one of infectious enterocolitis, and one of cellulitis). Eight serious events occurred in three patients from the placebo group (acute heart failure, bacterial vaginitis, chlamydia pelvic inflammatory disease, flare-up of lupus nephritis, pleural effusion, pericardial effusion, decreased serum albumin, and proteinuria). All of these events resolved. The two patients with acute myocardial infarction discontinued the study drug and recovered with routine therapy. The patient with infectious enterocolitis and the patient with cellulitis both recovered while study drug administration was continued.

Since it is difficult to assess the relation between infections and immunosuppressive treatment, all such events are shown in Table 4, but the incidence was similar in both groups. The major adverse event was nasopharyngitis in both groups, while events that only occurred in the tacrolimus group were viral gastritis, pyuria, vaginitis, folliculitis, and cellulitis (one case each). The common cold was the most frequent infection that occurred in both groups. During the study period, the serum concentration of tacrolimus ranged from 3.20 to 5.15 ng/mL, with a mean value of 4.35 ± 1.53 ng/mL.

Discussion

Lupus nephritis is a serious condition that can have a devastating impact on the quality of life if disease control is poor. The ultimate goal of medical treatment is to prevent renal failure. Because most patients do not show rapid progression to renal failure or hemodialysis and the course is protracted when progression occurs, clinical trials based on such true endpoints are difficult to perform. Accordingly, previous trials used the serum creatinine level, the frequency of exacerbation, daily urinary protein excretion, or other surrogate endpoints [23]. Among these endpoints, daily urinary protein excretion is a representative indicator of nephritis that was found to be one of the most important parameters in previous trials, because a decrease in proteinuria predicts a lower risk of progression to hemodialysis [24]. In contrast, it has been reported [25] that nephrotic syndrome is not a predictor of renal failure and that there is no association between daily protein excretion

and renal dysfunction [26], so proteinuria is not a definitive prognostic indicator for patients with lupus nephritis.

Lupus nephritis is an autoimmune disease, so indicators of immunological activity like autoantibodies and complement are considered to have clinical relevance. An association between autoantibody levels and the prognosis for renal function has been reported [27]. It has also been shown that higher doses of glucocorticoids decrease serious organ damage associated with SLE [28]. Furthermore, an association between complement levels and prognosis has been reported [29], and normalization of complement is related to improved renal function and a lower mortality rate due to SLE [30]. These reports suggest that autoantibodies and complement are important indicators of the prognosis of lupus nephritis. Therefore, to predict the long-term prognosis of lupus nephritis, such immunological parameters should be combined with parameters of nephritis, such as proteinuria and urinary sediment. Accordingly, we used the daily urinary protein excretion, the urinary RBC count, serum creatinine, anti-ds-DNA antibody, and complement (C3) to construct a comprehensive index of disease activity (the LNDAI) to assess the efficacy of tacrolimus.

At the final evaluation, the LNDAI was reduced by $32.9 \pm 31.0\%$ in the tacrolimus group and was increased by $2.3 \pm 38.2\%$ in the placebo group, showing a significant difference between the two groups ($p < 0.001$). Both proteinuria, a clinical feature of nephritis, and the complement (C3) level, an immunological parameter, showed significant differences between the two groups at most assessment times. In contrast, the urinary RBC count, serum creatinine, and anti-ds-DNA antibody level did not change markedly, and there were no significant differences between the groups with respect to the changes in these parameters after treatment, probably because baseline values were largely normal in both groups. Cyclosporine is an immunosuppressant used after transplantation in a similar manner to tacrolimus, and it is also employed for the treatment of nephrotic syndrome. Cyclosporine is thought to inhibit glomerular deposition of immune complexes by reducing the production of cytokines such as IL-2 by T cells, as well as by blocking T cell-mediated antibody production, thus reducing urinary protein excretion and the progression of nephritis [31]. In particular, it was recently reported that cyclosporine decreases proteinuria and protects the kidney via the regulation of cytoskeletal proteins in glomerular epithelial cells [32]. Cyclosporine differs from tacrolimus in that it exerts its immunosuppressive effect via cyclophilin, while tacrolimus acts via FKBP. However, both drugs are calcineurin inhibitors, so there is a possibility that tacrolimus acts via a mechanism similar to that of cyclosporine, which may have been a factor in the decrease of proteinuria.

In this study, the efficacy of tacrolimus at inducing remission was investigated in patients with moderately active nephritis and immune dysfunction, and a significant improvement was obtained compared with the effect of placebo. Accordingly, this suggests that tacrolimus could potentially be used as a remission inducer, not only in combination with steroids but also with other immunosuppressants for patients with higher disease activity.

The glucocorticoid dose showed little change during the study, probably because the protocol did not allow the dose to be increased again if tapering was performed. Further studies will be needed to determine whether tacrolimus therapy can reduce the use of glucocorticoids in patients with lupus nephritis.

Creatinine clearance was significantly lower in the tacrolimus group compared with the placebo group in week 12. However, the actual difference was about 20%, which is not so large. There was no significant difference between the groups with respect to the change in eGFR. Because serum creatinine did not change markedly and proteinuria improved, the risk of serious renal dysfunction due to tacrolimus therapy may not be very high. However, concern over aggravation of renal dysfunction remains, so careful observation would be needed during long-term administration, and further studies will also be required. In particular, the creatinine clearance should be monitored when tacrolimus is administered for a long period.

The percent change in the total SLEDAI score at the final evaluation showed a significant difference between the two groups, suggesting a beneficial effect of tacrolimus on the symptoms of SLE. However, the effect was not clear because few patients had any symptoms other than those of lupus nephritis at enrollment, so further studies will be needed to assess the efficacy of tacrolimus for SLE symptoms.

Serious cardiovascular adverse effects included two cases of acute myocardial infarction in the tacrolimus group and one case of acute heart failure in the placebo group. Both of the patients who developed acute myocardial infarction were men. One was a 47-year-old smoker with multiple risk factors (hypertension, hyperlipidemia, hyperuricemia, and a family history of ischemic heart disease). Myocardial infarction occurred on day 168 of administration. The other patient was 32 years old and had the risk factors of hyperlipidemia and obesity; myocardial infarction occurred on day 105 of administration. When the risk factors for coronary artery disease were compared, there were no significant differences between the two groups. Patients with SLE often have severe atherosclerosis, which is not fully explained by the Framingham risk factors [33], so some researchers have suggested that SLE itself may promote atherosclerosis [34]. Therefore, patients with lupus nephritis who have risk factors for coronary

artery disease should receive adequate treatment for these risk factors and careful monitoring during tacrolimus therapy.

In conclusion, tacrolimus was found to be a safe and effective (at least for 28 weeks) addition to glucocorticoid therapy for lupus nephritis in patients with persistent disease activity in whom dose reduction was difficult. Therefore, tacrolimus should be considered as one of the options for the treatment of lupus nephritis.

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