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Original Article

Clinical analysis of multi-target treatment for complex lupus nephritis

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Abstract: Objective: To observe the efficacy and safety of multi-target (tacrolimus + mycophenolate mofetil + prednisone) therapy for type III + V and IV + V type lupus nephritis. Methods: A total of 56 patients with lupus nephritis were randomly divided into a treatment group receiving multi-target treatment and a control group receiving intravenous cyclophosphamide combined with prednisone treatment, with 28 patients in each group. Clinical indicators and adverse reactions were observed before and 4, 12, 24, 48 and 72 weeks after treatment. Results: One patient withdrew from the treatment group and two patients from the control group due to adverse reactions within 72 weeks of treatment. Compared with those before treatment, urine protein quantification, ds-DNA antibody titer and systemic lupus erythematosus disease activity index (SLEDAI) scores were significantly decreased at 24 h after 72 weeks of treatment in both groups ($P < 0.05$). The total remission rate was 85.2% in the treatment group and 57.7% in the control group ($P < 0.05$) and dte total response rate was 59.3% and 30.8%, respectively ($P < 0.05$). Conclusion: Multiple target treatment of type III + V or IV + V type lupus nephritis has a higher total remission rate, a shorter treatment time, and a lower incidence of adverse reactions than cyclophosphamide and prednisone combined therapy.

Keywords: Tacrolimus, mycophenolate mofetil, cyclophosphamide, lupus nephritis

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease, and the most common and severe organ damage resulting from it is lupus nephritis (LN). The treatment of type III + V or IV + V LN is particularly difficult, and is often referred to as complex LN [1-3]. The treatment method for type III + V or IV + V LN patients is controversial. It is reported that the current recommended treatment of choice for LN is the National Institutes of Health (NIH) method, which combines glucocorticoids with intravenous cyclophosphamide (CYC). However, there is still high clinical inefficiency and a high recurrence rate. Some patients have poor prognosis, and easily relapse after drug withdrawal, especially for complex LN. Moreover, cyclophosphamide may induce or aggravate the infection, cause gonad damage, leukopenia, liver damage and other side effects, which all restricted its application [4-6]. Tacrolimus (FK506) can inhibit the production of IL-2 and IL-10, thereby inhibiting the proliferation of T lymphocytes and B lymphocytes, showing good efficacy in the treatment of lupus nephritis [7]. Mycophenolate Mofetil (MMF) selectively blocks T and B lymphocyte proliferation and inhibits antibody synthesis to achieve immunosuppressive effect [8]. A previous study reported that multi-target (tacrolimus + mycortisone + prednisone) treatment of complex LN has a high short-term solution rate and good safety [9]. However, there are few long-term observation studies on multi-target treatment. In this paper, we reported the efficacy of multi-target treatment for complex LN after being observed for up to 72 weeks.

Materials and methods

Study patients

From February 2012 to July 2017, 56 patients with complex LN were admitted to our hospital

and were selected for study. The research was conducted according to the principles of the World Medical Association Declaration of Helsinki. This study was approved by the ethical medical committee of the First Affiliated Hospital of Hainan Medical University. All subjects gave written informed consent. All patients had type III + V or type IV + V LN, according to the American rheumatism association (ARA) 1997 revised criteria for the diagnosis of SLE, and were given renal biopsy pathologic examination, with reference to the international society of nephrology/kidney pathological society with lupus nephritis (ISN/RPS) classification standards for classification (2003). Patients with severe infection, liver or kidney damage, severe hypertension or abnormal glucose metabolism were excluded from this study. This clinical research project was verified by the ethics committee of our hospital (20120337). All patients signed an informed consent before enrollment. According to a random number table method, 28 patients were divided into the treatment group, including 3 males and 25 females. They were aged 18 to 46 years, with a median of age 31, and an average of (31.2±9.3) years; the course of disease was 1 to 13 months, with a median of 6 months, and an average of (6.1 + 5.8) months, including 13 type III + V cases, and 15 type IV + Vcases. The control group consisted of 28 patients, including 3 males and 25 females. They were aged 18 to 43 years, with a median of 30 years, and an average of (30.6-8.7) years; the course of disease was 1 to 12 months, with a median of 6 months, and an average of (5.9 + 6.1) months, including 12 type III + V cases, and 16 type IV + V cases.

In the treatment group, 1 patient withdrew from the study due to adverse reactions, and the final number was 27. In the control group, 2 patients withdrew from the study due to adverse reactions, and the actual number of patients treated was 26. The 3 patients who withdrew were not included in the comparison of efficacy, but the adverse reactions were counted. There were no statistically significant differences between the two groups in the course of disease, SLEDAI score, urine protein quantification at 24 h, renal function, serum albumin (ALB), anti-ds-DNA antibody, complement C3, C4 and pathological type distribution ($P > 0.05$).

Methods

Treatment group: Tacrolimus capsule (FK506, Tacrolimus, 1 mg/tablet, 50 tablets/box, Hangzhou Zhongmei Huadong Pharmaceutical Co., LTD.) was used at 0.06-0.08 mg/(kg·d). Mycophenolate mofel dispersible tablets (MMF, Trade name: Saikepine, 0.25 g/tablet, 40 tablets/box, Hangzhou Zhongmei Huadong Pharmaceutical Co., LTD.) was used at 20-30 mg/(kg·d). Prednisone tablet was used at 0.6~0.8 mg/(kg·d). FK506 and MMF were taken twice, and prednisone was taken in the morning. The dose was reduced gradually after 6-8 weeks. FK506 was reduced to ≤ 0.04 mg/(kg·d) for 4-5 months, and then slowly decreased to 1 mg/d. MMF was reduced to ≤ 10 mg/(kg·d) for 4-5 months, and then slowly decreased to 250 mg/d. Prednisone was reduced to ≤ 0.4 mg/(kg·d) after 4~5 months, and then slowly decreased to 10 mg/d. The total course of treatment was 72 weeks.

Control group: According to the NIH protocol, patients received intravenous cyclophospsamide shock with a dose of 0.5-0.75 g/m once a month, 6 times in total. After 6 months of treatment, the CYC dose remained unchanged, but was administered once every 3 months, and the total course of treatment was 72 weeks. Prednisone was used in the same way as in the treatment group. Other types of immunosuppressive agents were not used during treatment either both group.

Follow-up observation

Patients were observed every 4 weeks before and after treatment until 72 weeks, including routine urine, urine protein quantification at 24 h, ALB, blood creatinine (SCr), blood urea nitrogen, anti-ds-DNA antibody, complement C3, complement C4, anti-nuclear antibody (ANA), liver function, renal function, routine blood, blood glucose, and SLEDAI score. The occurrence of adverse reactions was recorded.

Among them, the assessment of SLEDAI-2000 score for SLE patients: 0-4 was classified as basically inactive, 5-9 as mild activity, 10-14 as moderate activity, and ≥ 15 as severe activity.

According to the guidelines for the treatment of renal diseases, the clinical efficacy of lupus nephritis was divided into complete response (CR), partial response (PR) and no response

Table 1. Comparison of biochemical indexes between the two groups before and after treatment (mean ± SD)

Group	Time	Urine protein (g/24 h)	ALB (g/L)	C3 (g/L)	SCr (μmol/L)
Treated	0	3.8±2.7	22.8±15.6	0.45±0.37	83.9±40.6
	4 w	2.1±1.9	29.8±12.9	0.57±0.31	82.1±32.9
	12 w	1.5±1.5	31.6±12.7	0.62±0.28	72.3±37.6
	24 w	1.0±1.2	36.2±9.8	0.69±0.27	70.6±38.5
	48 w	0.9±1.2	40.1±10.3	0.81±0.26	58.7±33.2
	72 w	0.5±1.2	42.3±10.3	0.92±0.31	56.7±32.1
control	0	3.7±2.9	23.2±16.1	0.46±0.38	84.1±41.3
	4 w	3.3±2.92	24.5±10.6	0.47±0.32	82.1±39.2
	12 w	2.2±1.4	25.2±11.2	0.50±0.34	79.2±40.7
	24 w	1.8±1.8	30.6±12.4	0.63±0.35	78.6±38.2
	48 w	1.5±1.9	32.1±12.2	0.70±0.29	76.3±35.8
	72 w	1.3±1.1	35.8±11.6	0.81±0.23	72.5±32.5
F_{time}		1.252	5.552	2.536	65.966
P_{time}		< 0.001	< 0.001	< 0.001	< 0.001
F_{group}		12.946	12.792	0.274	5.828
P_{group}		0.001	0.001	0.001	0.019
$F_{interaction}$		77.172	1.346	57.053	12.047
$P_{interaction}$		< 0.001	< 0.001	< 0.001	< 0.001

(NR). CR: Quantitative urine protein < 0.4 g/24 h, urine RBC < 3/HP, no WBC or tubular shape, normal ALB, normal SCr, anti-ds-DNA antibody turned negative. PR: at 24 h, urine protein decreased by ≥ 50%, but > 0.4 g; ALB ≥ 30 g/L but still not normal, SCr decreased by ≥ 50% but not to normal. The titer of anti-ds-DNA antibody was significantly decreased. NR: urinary protein quantity > 2.0 g/24 h or 50% decreased, ALB < 30 g/L, SCr increased > 50%, and without significant decrease in titer of anti-ds-DNA antibody. The sum of CR and PR was calculated as the total mitigation rate.

Statistical analysis

SPSS 19.0 statistical software was used. Quantity data were expressed as mean ± standard deviation. ANOVA was used for comparison of repeated measures between groups. The numeration data was represented by n (%), and the chi-squared test was performed. P < 0.05 indicated that the difference was statistically significant.

Results

Clinical therapeutic effect

The urinary protein level in the treatment group and the control group decreased at 24 h at 4, 12, 24, 48 and 72 weeks after treatment, while ALB level increased (P < 0.05). The indexes of the treatment group were better than those of the control group (P < 0.05), as shown in **Table 1**.

Remission rate

The total remission rate of the treatment group at 4, 12, 24, 48 and 72 weeks was higher than that of the control group (P < 0.05), and the complete remission rate of the treatment group at 72 weeks was higher than that of the control group (P < 0.05), as shown in **Table 2**.

Comparison of the positive rate of anti-ds-DNA antibody between the two groups

After 72 weeks of treatment, the positive rate of ds-DNA antibodies in the two groups decreased compared with that before treatment (P < 0.05). See **Table 3**.

Comparison of SLEDAI scores between the two groups after 72 weeks of treatment

SLEDAI scores decreased compared with those before treatment (P < 0.05). SLEDAI score of the treatment group was lower than that of the control group (P < 0.05). See **Table 4**.

Adverse reactions

The incidence of adverse reactions in the study group was lower than that in the control group (χ^2=6.171, P=0.013), see **Table 5**.

Discussion

LN is one of the main complications and death factors of SLE. The pathogenesis of LN is not fully understood. More and more biomarkers have been discovered and the treatment regimens are more diversified [10]. The traditional treatment of LN is mainly glucocorticoids combined with cyclophosphamide, but the therapeutic effect of complex LN is poor, and the incidence of adverse reactions is high [11-13].

This study observed the efficacy of multi-target therapy for complex LN compared with conven-

Table 2. Comparison of total remission rates between the two groups at 4, 12, 24, 48 and 72 weeks of treatment [n (%)]

Group	Time	CR	PR	NR	Total rate
Treated (n=27)	4 w	2 (7.4)	8 (29.6)	17 (63.0)$^{\Delta}$	10 (37.0)$^{\Delta}$
	12 w	7 (25.9)$^{\Delta}$	8 (29.6)	12 (44.4)$^{\Delta}$	15 (55.6)$^{\Delta}$
	24 w	11 (40.7)$^{\Delta}$	7 (25.9)	9 (33.3)$^{\Delta}$	18 (66.7)$^{\Delta}$
	48 w	12 (44.4)	8 (29.6)	7 (25.9)$^{\Delta}$	21 (77.8)$^{\Delta}$
	72 w	16 (59.3)$^{\Delta}$	6 (22.2)	5 (18.5)$^{\Delta}$	23 (85.2)$^{\Delta}$
control (n=26)	4 w	0 (0.0)	2 (7.7)	24 (92.3)$^{\Delta}$	2 (7.7)$^{\Delta}$
	12 w	0 (0.0)$^{\Delta}$	6 (23.1)	20 (76.9)$^{\Delta}$	6 (23.1)$^{\Delta}$
	24 w	3 (11.5)$^{\Delta}$	7 (26.9)	16 (61.5)$^{\Delta}$	10 (38.5)$^{\Delta}$
	48 w	6 (23.0)	7 (26.9)	13 (50.0)$^{\Delta}$	13 (50.0)$^{\Delta}$
	72 w	7 (26.9)$^{\Delta}$	8 (30.8)	10 (38.5)$^{\Delta}$	15 (57.7)$^{\Delta}$

Note: Comparison of treatment group and control group was at the same observation time point, $^{\Delta}P < 0.05$.

Table 3. Comparison of ds-DNA positive rates between the two groups at 72 weeks of treatment [n (%)]

Group	Before	after 72 w	χ^2	P
Treated (n=27)	25 (92.6)	6 (22.2)	23.29	0.000
Control (n=26)	24 (92.3)	9 (34.6)	18.660	0.000
χ^2	0.02	1.003		
P	0.969	0.317		

Table 4. Comparison of SLEDAI scores between the two groups before and after 72 weeks of treatment ($\bar{x}\pm s$)

Group	before	after 72 w	t	P
Treated (n=27)	12.3±5.8	1.3±1.1	9.682	0.000
Control (n=26)	12.5±6.1	3.9±2.3	6.727	0.000
t	0.122	5.218		
P	0.903	0.026		

tional cyclophosphamide and prednisone. We found that 4, 12, 24, 48 and 72 weeks after treatment, the total response rate was 37.0%, 55.6%, 66.7%, 77.8%, 85.2%, higher than that of control group, which were 7.1%, 23.1%, 38.5%, 50.0%, 57.7%. For each observation time point, the patient's level of urinary protein, serum creatinine were lower than the control group; blood albumin and complement levels were higher than in the control group. Rapid control in patients with proteinuria in the treatment group effectively prevented further damage of continuous proteinuria to the kidneys in lupus patients, improved the blood albumin and ameliorated the effect of lupus more quickly. Moreover, this study showed that the CR rate of patients in the study group was 59.3% after 72 weeks of treatment, which was significantly higher than that of the control group (30.8%). SLEDAI score was lower than that of the control group, and adverse reactions in the study group were less than those in the control group, with mild adverse reactions and good safety. Common adverse reactions were infection, liver damage and blood glucose increase, which could be controlled by symptomatic treatment.

Tacrolimus is a neurocalcalin inhibitor, which can inhibit the transcription process of lymphoid genes and exert a strong immunosuppressive effect by inhibiting both cellular and humoral immune mechanisms [14, 15]. It is reported that tacrolimus combined with methylprednylone is effective in the treatment of LN syndrome with high clinical remission rate [16]. Mycophenolate mofetil can selectively block T and B lymphocyte proliferation, inhibit antibody synthesis, and block the formation of endothelial adhesion factors, as well as inhibition of arterial smooth muscle cells and endothelial cell proliferation. Therefore, Mycophenolate mofetil has advantages in the treatment of vascular inflammatory lesions. Multi-target treatment can block more disease pathogenic factors, which can overcome the limitations of many single target drugs. Multi-target drug treatment can also adjust the network system of multiple links, produce synergistic effects and achieve the best treatment effect without easily developing resistance. These methods have been applied in the treatment of many major diseases [17-20]. The complex form of LN often has abnormal cellular immunity and humoral immunity, and different degrees of vasculitis; Tacrolimus + Mycophenolate mofetil + prednisone with multiple targets can be integrated for the treatment of a variety of immune reactions of LN tissue damage. This method can enhance the curative effect and reduce the use of cytotoxic drugs.

Table 5. Adverse reactions in the two groups [n (%)]

Group (n=number of cases)	Lung infection	Transaminase elevated	diarrhea	Nausea/ vomiting	Leukocytosis	Herpes zoster	Menstrual disorders	Hair loss	Elevated blood sugar	Total
Treatment group (n=28)	1 (3.6)	1 (3.6)	1 (3.6)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.2)	6 (21.4)
Control group (n=28)	1 (3.6)	2 (7.2)	1 (0.0)	3 (10.8)	2 (7.2)	1 (3.6)	2 (7.2)	2 (7.2)	1 (3.6)	15 (53.5)

Above all, Tacrolimus + Mycophenolate mofetil + prednisone has multiple targets for treatment of type III + V or type IV + V LN and the remission rate is significantly higher than cyclophosphamide combined therapy with prednisone, and adverse reactions are relatively rare, lighter, and can be improved by symptomatic treatment.

This study has some deficiencies. First, the sample size of this study is relatively small, and it is a single-center study, so the efficacy of multi-target treatment for complex lupus still needs to be further expanded to be carried out in other treatment centers with larger samples and randomized controlled trials, so as to obtain more powerful and credible research evidence. Secondly, this study failed to observe the long-term prognosis, progression to end-stage renal disease, and recurrence of LN in the two groups of patients. Prospective studies with larger samples are still needed to conduct long-term clinical observation on patients to observe the effect of multi-target treatment on long-term prognosis of LN.

Disclosure of conflict of interest

None.

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