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【 ORIGINAL ARTICLE 】

Efficacy and Safety of Hydroxychloroquine Therapy for Systemic Lupus Erythematosus Patients Depend on Administration Dose

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Abstract:

Objective Hydroxychloroquine (HCQ) has been prescribed in Japan only relatively recently and is recommended for the treatment of skin lesions, arthritis and renal lesions according to the Japanese Guideline for the Management of systemic lupus erythematosus (SLE) (2019). However, the associations between the efficacy and safety and the HCQ dose in Japanese SLE patients remain unclear. We investigated the efficacy and safety of different HCQ doses in Japanese SLE patients with a low disease activity who were not receiving immunosuppressants.

Methods The disease activity was evaluated using the SELENA-SLEDAI 2011 criteria, the Cutaneous Lupus Erythematous Disease Area and Severity Index (CLASI) and serum biomarkers. Safety was evaluated via the frequency of adverse events over a period of three months.

Results We enrolled 61 SLE patients treated with HCQ and no additional immunosuppressive therapy for more than 3 months. HCQ was administered to 46 patients at the usual dose and to 15 cases at a lower than usual dose. Although the CLASI activity scores decreased significantly in both groups, the magnitude of this decrease was larger in the usual-dose HCQ group than in the low-dose HCQ group. SLEDAI scores and immunological activity were significantly improved only in the usual-dose HCQ group. In addition, changes in the serum complement levels in the usual-dose HCQ group were more dramatic than in the low-dose HCQ group six months after the initiation of HCQ administration. Adverse events were more frequent in the usual-dose HCQ group than in the low-dose HCQ group (30.4% and 13.3%, respectively).

Conclusion HCQ therapy is effective for maintenance therapy of SLE patients. The usual dose of HCQ may have some advantage in ameliorating low complement levels.

Key words: systemic lupus erythematosus, hydroxychloroquine, skin lesion

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by inflammation in various organs and is intimately associated with both the innate and adaptive immune systems. Hydroxychloroquine (HCQ) is effective for treating cutaneous lupus erythematosus (CLE) and SLE symptoms, such as rashes, joint pain and fatigue (1). According to the Japanese Guideline for the Management of SLE (2019), HCQ is recommended for the treatment of skin lesions, arthritis and renal lesions (2). In addition, HCQ can prevent disease flare in SLE patients and improve survival rates. The European League against Rheumatism recom-

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Table 1. Characteristics of SLE Patients Treated with Usual-dose or Low-dose HCQ.

	Normal Range	Usual-dose HCQ	Low-dose HCQ	p value*
		n=46†	n=15	
Age, years, mean±SD		40.3±12.4	46.1±9.3	0.069
Female, No.(%)		43 (93)	13 (87)	0.404
Disease duration, years, mean±SD		13.2±10.1	11.1±7.8	0.609
HCQ dose per body weight, mg/kg, mean±SD		5.1±1.2	3.9±0.6	**0.0004**
Disease characteristics				
Skin lesions (photosensitivity), No.(%)		40 (87)	14(93)	0.479
Renal lesions, No.(%)		22 (48)	1 (7)	**0.0043**
SLEDAI score, mean±SD		3.9±2.2	2.7±1.8	0.058
anti-dsDNA, IU/mL, mean±SD	0-12.0	14.8±16.8	9.9±3.1	0.906
C3, mg/dL, mean±SD	68-144	79.3±24.0	86.9±26.0	0.321
C4, mg/dL, mean±SD	12-33	16.1±7.6	19.2±9.2	0.296
CH50, U/mL, mean±SD	30-50	33.6±9.6	37.3±7.4	0.293
White blood cell count per μL, mean±SD	4,700-8,700	5,090.0±1,806.9	6,888.7±3,217.0	**0.015**
Lymphocyte count per μL, mean±SD		1,066.1±599.0	1,136.7±570.0	0.711
Platelet count, $\times 10^4$ per μL, mean±SD	15-35	22.5±6.7	23.3±5.3	0.589
Cr, mg/dL	M:0.7-1.3 F:0.5-1.0	0.63±0.19	0.65±0.16	0.456
eGFR, mL/min/1.73m^2	15-35	93.0±29.2	85.2±19.3	0.299
CLASI activity‡, mean±SD		3.6±3.2	2.6±2.8	0.108
Treatment				
Prednisone				
No.(%)		41 (89)	15 (100)	0.114
Median Dose, mg/day (range)		5 (1-15)	7 (2-20)	
Tacrolimus, No.(%)		16 (46)	8 (50)	
Mycophenolate mofetil, No.(%)		9 (26)	1 (6)	
Cyclosporine A, No.(%)		4 (6)	3 (19)	
Mizoribine, No.(%)		2 (6)	0 (0)	
Methotrexate, No.(%)		2 (6)	3 (19)	
Azathioprine, No.(%)		2 (6)	1 (6)	

Cr: creatinine, eGFR: estimated glomerular filtration rate, SD: standard deviation

†Five cases were switched to low-dose HCQ because of adverse events.

‡Active skin involvement was present in 34 patients and 12 patients in the usual- and low-dose HCQ groups, respectively.

* p values from chi-square tests or Wilcoxon rank sum tests.

mended in 2019 that all SLE patients receive HCQ at a dose not exceeding 5 mg/kg real body weight (3).

The pharmacologic management of HCQ and the optimal daily HCQ dose for treating SLE are controversial (4, 5). HCQ should be administered at a dose of ≤ 6.5 mg/kg ideal body weight to prevent ocular toxicity (6, 7). However, low blood HCQ concentrations are associated with increased SLE disease activity and are a strong predictor of disease exacerbation (8). In a randomized controlled trial of HCQ treatment for SLE patients with stable active disease, patients with blood HCQ levels ≥1,000 ng/mL had a reduced frequency of SLE flares over a 7-month period (9). Recently, 1 study suggested a target blood HCQ level of >0.6 mg/L (600 ng/mL) to reduce the risk of renal flares in patients with lupus nephritis (10).

After HCQ was approved for SLE treatment in Japan in July 2015, many SLE patients receiving immunosuppressants began to be additionally treated with HCQ. Several studies demonstrated the effectiveness of HCQ in Japanese patients, which permitted the reduction of the corticosteroid dose (11, 12). However, few studies explored the effect of the dose on the efficacy and safety of HCQ for Japanese SLE patients.

The present study therefore assessed the relationships among the HCQ dose, safety and efficacy during the maintenance phase of SLE.

Materials and Methods

Patients

This was a multi-center, retrospective study. All SLE patients enrolled in this study were diagnosed using the American College of Rheumatology criteria (13) or SLE International Collaborating Clinics criteria (14) and treated with HCQ. Patients were routinely followed up in our institute, Utazu Hospital and Tamamo Clinic from September 2015 to December 2017. All patients were ≥14 years old

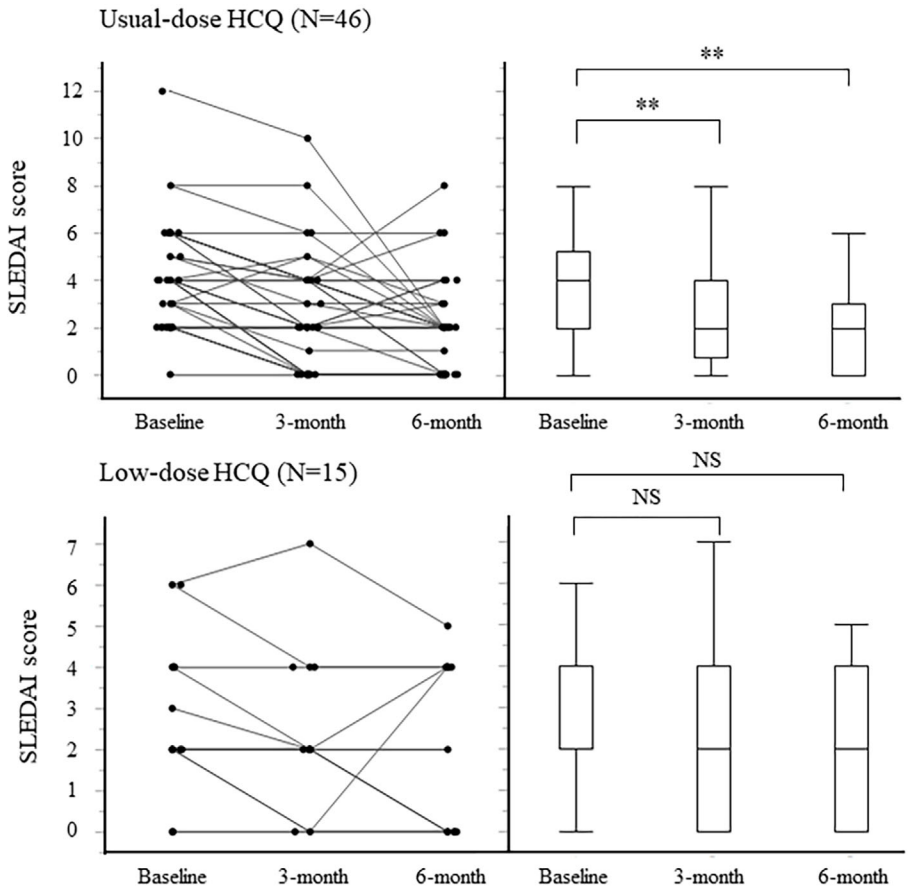

Figure 1. SLEDAI scores at baseline, 3-month follow-up and 6-month follow-up in SLE patients treated with usual-dose HCQ or low-dose HCQ. SLEDAI scores decreased significantly in patients treated with the usual dose of HCQ after 3 months' treatment. For statistical analyses: **p<0.001. P values from Wilcoxon's rank signed test.

and had been receiving oral HCQ sulfate (Plaquenil; Sanofi-Winthrop, Paris, France) for at least 3 months.

Because all patients in the maintenance phase of SLE were included, patients who required additional glucocorticoid or immunosuppressive treatment within three months of starting HCQ or discontinued HCQ within a period of three months were excluded. Informed consent was obtained from all participants. The study was approved by the ethics committee of Kagawa University.

The usual HCQ dose was based on the ideal body weight (calculated using the modified Broca's method): 200 mg daily for patients with an ideal body weight of <46 kg; 200 and 400 mg on alternate days for a weight of 46 kg to <62 kg; and 400 mg daily for a weight of ≥62 kg.

Alternatively, some patients received low-dose HCQ (200 mg daily) at the discretion of the attending physician despite having an ideal weight ≥46 kg.

Outcome measures

- **Efficacy**

The disease activity was measured using the SELENA-SLEDAI 2011 tool. The cutaneous disease activity was evaluated using the Cutaneous Lupus Erythematous Disease Area and Severity Index (CLASI). The immunological activity was evaluated via serum levels of complement factors (C3, C4 and CH50), anti-double stranded (ds) DNA antibodies and counts of white blood cells, lymphocytes and platelets.

- **Safety**

Safety endpoints included adverse events (AEs), serious AEs, laboratory test values (e.g., serum creatinine levels and estimated glomerular filtration rate) and vital signs. AEs were defined by a physician's assessment. Each ocular AE was assessed by an ophthalmologist.

Statistical analyses

Outcomes in patients treated with usual-dose HCQ and low-dose HCQ were compared using chi-square test or Fisher's exact test for categorical variables and using Student's *t*-test or Wilcoxon's rank signed test (for non-normally distributed data) for continuous variables. All p values were two-sided. Values of $p<0.05$ were considered statistically significant. In all statistical analyses, we used the following abbreviations: *p<0.05, **p<0.01, ***p<0.001. Data were analyzed using the JMP® 13 software program (SAS Institute, Cary, USA).

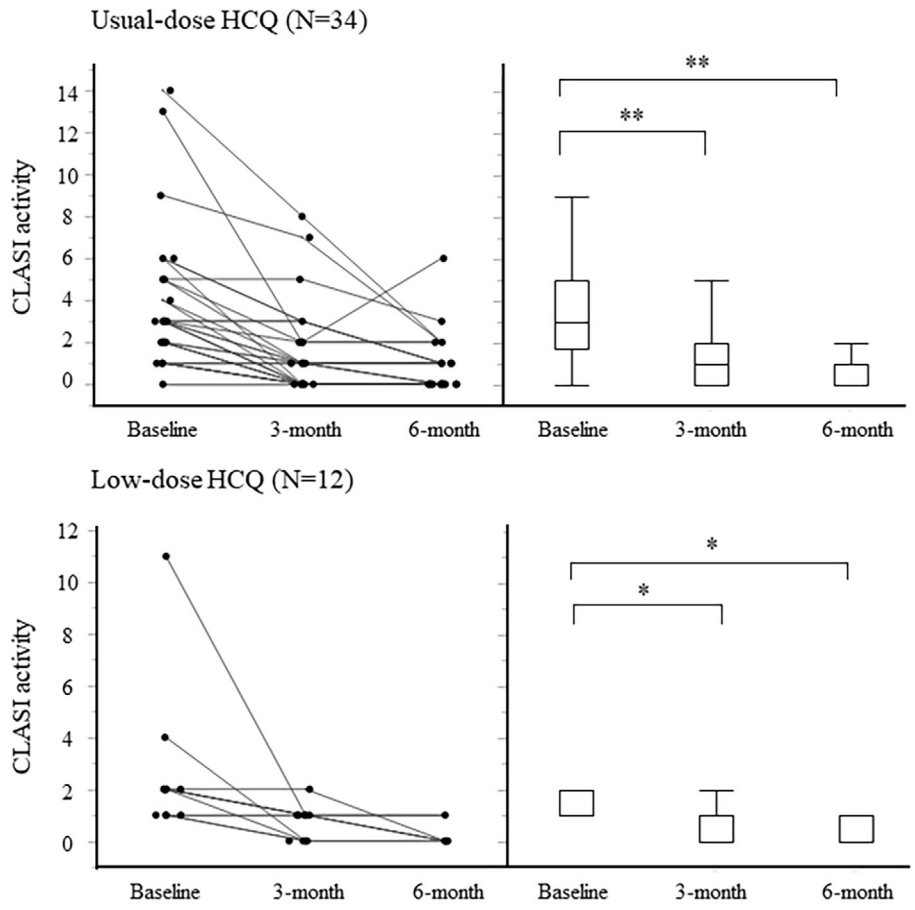

Figure 2. CLASI activity scores at baseline, 3-month follow-up and 6-month follow-up in SLE patients treated with usual-dose HCQ or low-dose HCQ. For statistical analyses: *p<0.0167, **p<0.001. P values from Wilcoxon's rank signed test.

Results

Of the 77 patients who started HCQ therapy, 66 were in the maintenance phase of SLE. Five patients receiving the usual dose of HCQ (based on ideal body weight) discontinued therapy within a period of 3 months because of AEs (rash, n=3; stomachache, n=1; dizziness, n=1). The HCQ retention rate was 90% at the usual dose and 100% at the lower dose.

The clinical characteristics and disease activities of the 61 SLE patients enrolled in this study are shown in Table 1. HCQ was administered to 48 patients at the usual dose and to 15 patients at a lower dose. The occurrence of renal lesions was higher and the counts of white blood cells significantly lower in the usual-dose HCQ group than in the low-dose HCQ group. There were no other significant differences in any characteristics between the two groups at baseline.

Association between HCQ dose and efficacy

The CLASI activity scores and SLEDAI scores of the usual-dose and low-dose HCQ groups are shown in Figs. 1 and 2, respectively. The CLASI activity scores and SLEDAI scores decreased significantly in all patients after 3 months' HCQ treatment (mean CLASI activity score change -2.0, p<0.0001; mean SLEDAI score change -1.0, p< 0.0001). Although both groups showed significant reductions in CLASI activity scores, the magnitude of this decrease was larger in the usual-dose HCQ group than in the low-dose HCQ group (mean CLASI activity score changes -2.2, p<0.0001 and -1.8, p=0.0439, respectively).

The SLEDAI scores decreased in patients treated with both doses of HCQ, but this reduction only achieved statistical significance in the usual-dose HCQ group after 3 months' treatment (mean SLEDAI score change -1.3, p< 0.0001).

Changes in serum anti-dsDNA antibody levels (IU/mL), C3 levels (mg/dL), C4 levels (mg/dL) and CH50 levels (U/mL) from baseline to 3-month and 6-month follow-up are shown in Table 2. Patients treated with the usual dose of HCQ had significantly decreased serum levels of anti-dsDNA antibodies at 3-month follow-up (mean change -3.7 IU/mL, p=0.0012) and increased serum levels of C3 and CH50 at 6-month follow-up (mean change 3.8 mg/dL, p= 0.014 and 1.9 U/mL, p=0.016, respectively). However, patients treated with low-dose HCQ showed no significant changes in any of these parameters, even at 6-month follow-

Table 2. Impact of HCQ Doses on SLE Disease and Immunological Activity.

Usual-dose HCQ (n=46)						
	Normal Range	**Baseline**	**3-month follow-up**	**6-month follow-up**	**0-3 month p value***	**0-6 month p value***
SLEDAI score, mean±SD		3.9±2.2	2.7±2.2	2.2±2.4	**<0.0001**‡	**<0.0001**‡
anti-dsDNA antibodies, IU/mL, mean±SD	0-12.0	14.8±16.8	11.3±14.1	11.9±13.9	**0.0012**‡	0.0170
C3, mg/dL, mean±SD	68-144	79.3±24.0	82.5±23.2	83.1±22.0	0.026	**0.014**‡
C4, mg/dL, mean±SD	12-33	16.1±7.6	16.9±6.9	17.7±7.5	0.086	0.018
CH50, U/mL, mean±SD	30-50	33.6±9.6	35.1±8.8	35.5±9.1	0.038	**0.016**‡
White blod cell count per μL, mean±SD	4,700-8,700	5,090.0±1,806.9	5,327.2±1,953.0	5,097.6±1,558.1	0.308	0.922
Lymphocyte count per μL, mean±SD		1,066.1±599.0	1,169.4±690.8	1,208.2±725.3	0.065	0.048
Platelet count, $\times 10^4$ per μL, mean±SD	15-35	22.5±6.7	22.9±6.0	22.9±6.4	0.392	0.627
CLASI activity†, mean±SD		3.6±3.2	1.4±2.0	0.8±1.3	**<0.0001**‡	**<0.0001**‡

Low-dose HCQ (n=15)					
	Baseline	**3-month follow-up**	**6-month follow-up**	**0-3 month p value***	**0-6 month p value***
SLEDAI score, mean±SD	2.7±1.8	2.2±1.9	2.2±1.9	0.094	0.012
anti-dsDNA antibodies, IU/mL, mean±SD	9.9±3.1	9.3±3.8	9.4±4.9	1.000	0.750
C3, mg/dL, mean±SD	86.9±26.0	84.1±26.6	87.8±27.3	0.401	0.045
C4, mg/dL, mean±SD	19.2±9.2	18.4±8.1	17.7±8.5	0.557	0.025
CH50, U/mL, mean±SD	37.3±7.4	37.1±6.9	33.5±5.8	0.922	0.039
White blod cell count per μL, mean±SD	6,888.7±3,217.0	7,235.3±2,375.2	6,294.3±1,700.1	0.525	0.916
Lymphocyte count per μL, mean±SD	1,136.7±570.0	1,149.6±704.9	1,282.1±750.9	1.000	0.502
Platelet count, $\times 10^4$ per μL, mean±SD	23.3±5.3	21.8±5.9	22.2±6.3	0.296	0.594
CLASI activity†, mean±SD	2.6±2.8	0.8±0.6	0.4±0.5	**0.0039**‡	**0.0078**‡

SLEDAI score, serum biomarkers and CLASI activity scores at baseline were compared with these biomarkers after 3 and 6 months of HCQ treatment with Bonferroni correction.

Patients treated with the usual dose of HCQ had significantly improved serum levels of anti-dsDNA antibodies and serum levels of C3 and CH50. while patients treated with low-dose HCQ showed no significant changes in any of these parameters.

All values are presented as means+/- standard deviations (SD) unless otherwise indicated.

* p values from Student's t-tests or Wilcoxon rank signed tests.

‡p<0.0167.

†Active skin involvement was in thirty-four patients and in twelve patients, respectively.

up (dsDNA antibody levels, -0.5 IU/mL, p=0.75; C3 levels, 0.9 mg/dL, p=0.045; CH50 levels, -3.8 U/mL, p=0.039).

No significant changes in counts of white blood cells, lymphocytes or platelets were observed in either group from baseline to 3-month and 6-month follow-up. Thus, the serum levels of complement factors and anti-dsDNA antibodies were significantly ameliorated only in patients treated with the usual dose of HCQ.

Next, we compared the magnitudes of changes in the CLASI activity scores, SLEDAI scores and immunological biomarkers between the usual-dose HCQ group and low-dose HCQ group over the six-month period following the initiation of HCQ therapy. The magnitudes of changes in the SLEDAI score, CLASI activity score and serum anti-dsDNA antibody levels were higher in the usual-dose HCQ group than in the low-dose HCQ group; however, this difference was not statistically significant (SLEDAI score, p=0.104; CLASI activity score, p=0.367; anti-dsDNA antibody level, p=0.516; Fig. 3A-C). Conversely, the changes in serum complement levels in the usual-dose HCQ group were significantly more dramatic than in the usual-dose HCQ group (C3, p=0.0041; C4, p=0.0008; CH50, p=0.0127; Fig. 3D-F).

AEs

AEs are shown in Table 3. Overall, AEs occurred in 16/61 patients (26.2%). The most common AE was diarrhea, which occurred exclusively in patients treated with the usual dose of HCQ. Ocular symptoms occurred in one patient

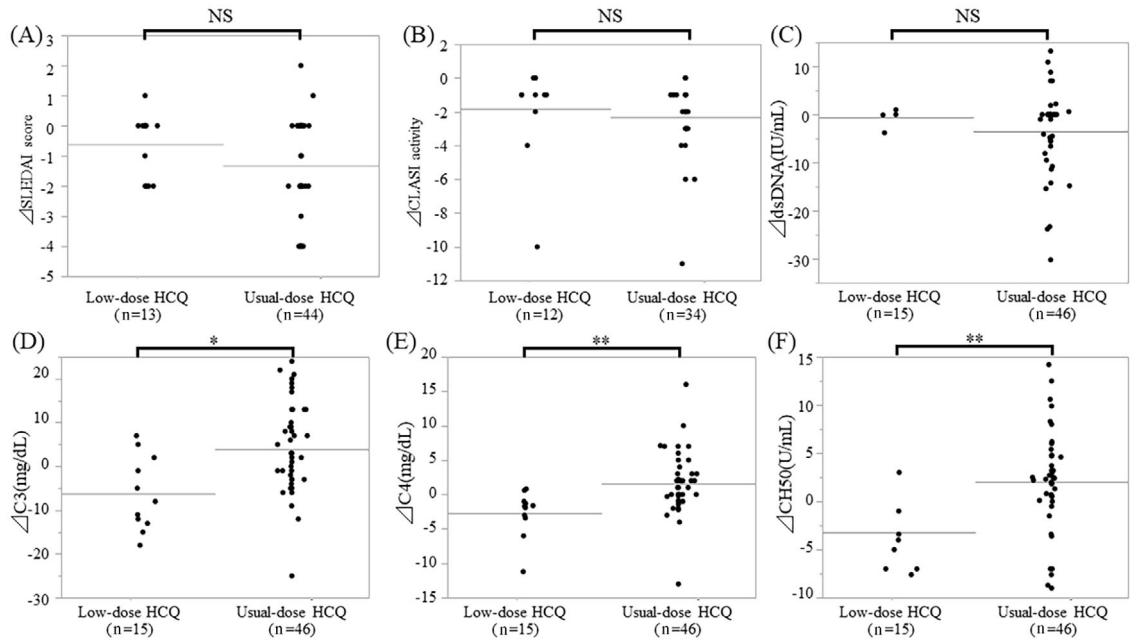

Figure 3. Magnitudes of changes in SLEDAI scores (A), CLASI activity scores (B) and serum immunological biomarker levels (C-F) in SLE patients treated with usual-dose HCQ or low-dose HCQ at 6 months after the initiation of HCQ therapy. The magnitudes of changes in serum complement levels (D-F) in the usual-dose HCQ group were significantly higher than those in the low-dose HCQ group. For statistical analyses: *p<0.05, **p<0.001, and NS: not significant. P values from Wilcoxon's rank sum test.

Table 3. Frequency of Adverse Events in SLE Patients Treated with Usual-dose or Low-dose HCQ.

Adverse Event	Usual-dose HCQ (n=46)	Low-dose HCQ (n=15)	p value
Diarrhea, No. (%)	10 (22)	0	0.055
Rash, No. (%)	3 (7)	1 (7)	1.000
Malaise, No. (%)	2 (4)	1 (7)	1.000
Eye symptom	0	1 (7)	0.246
visual field defect, No. (%)	1 (2)	0	1.000
color vision defect, No. (%)			
Fever, No. (%)	2 (4)	0	1.000
Pericarditis, No. (%)	1 (2)	0	1.000

The frequency of adverse events was lower in the low-dose HCQ group than in the usual-dose HCQ group, but this difference was not statistically significant.
p values from Fisher's exact tests.

treated with the usual dose of HCQ and one patient treated with low-dose HCQ. Both patients were confirmed to have no ocular abnormalities by an ophthalmologist. One patient in the usual-dose HCQ group developed pericarditis and was hospitalized and treated with steroid mini-pulses.

The frequency of AEs was lower in the low-dose HCQ group than in the usual-dose HCQ group (13.3% and 30.4%, respectively), but this difference was not statistically significant.

Discussion

This study demonstrated that CLE improved significantly in SLE patients following 3 and 6 months' treatment with both the usual dose and a lower dose of HCQ. The magnitude of CLASI activity score changes was higher in SLE patients treated with the usual dose of HCQ than in those treated with low-dose HCQ, although this difference was not statistically significant. Only patients treated with the usual dose of HCQ showed improvements in disease activity following treatment.

Previous studies of HCQ therapy for SLE suggested that a critical threshold of HCQ dose and HCQ blood concentration was required for efficacy. Some reports suggested that HCQ levels above 1,000 ng/mL were required to reduce SLE flares, although these findings did not extend to a daily

HCQ dose. Although low blood HCQ concentrations are associated with active SLE disease, our study showed that low-dose HCQ is effective in ameliorating CLE during the maintenance phase of SLE. However, skin lesions are not always associated with SLE disease activity.

Fortunately, no patients developed SLE flares during the three-month follow-up period in this study. This finding may have two potential explanations. First, all patients had inactive SLE, and skin lesions were the only symptoms of most patients. Second, the follow-up period was only three months, so some patients may have developed SLE flares over a longer period (such as six months, as in our previous study).

However, five patients in the usual-dose HCQ group were switched to a lower HCQ dose due to AEs occurring within the follow-up period. These five patients did not affect the major results of our study: that patients treated with the usual dose of HCQ had significantly decreased CLASI activity scores, SLEDAI scores and serum levels of anti-dsDNA antibodies, C3 and CH50 after three months' treatment. The CLASI activity scores and anti-dsDNA antibody levels of all five cases switched from usual-dose to low-dose HCQ also decreased.

Our data also showed that treatment with the usual dose of HCQ was associated with increased risk of AEs compared with low-dose HCQ. The most common AEs in the usual-dose HCQ group were gastrointestinal reactions, especially diarrhea. However, the symptoms of 3 of 10 patients who developed diarrhea in the usual-dose HCQ group improved after they were switched to a decreased HCQ dose. A previous study showed that a high HCQ dose was associated with the risk of gastrointestinal AEs in rheumatoid arthritis patients (15). Although the high HCQ dose used to treat rheumatoid arthritis patients was more than twice the usual dose used in our study, SLE patients treated with low-dose HCQ did not experience diarrhea. Thus, the HCQ dose appeared to correlate with risk of gastrointestinal AEs in this study.

The five patients who stopped treatment with usual-dose HCQ over the three-month follow-up period due to AEs were excluded. The most common AEs were rash (three patients), stomachache (one patient) and dizziness (one patient). No significant difference was observed in the frequency of AEs in patients treated with usual-dose or low-dose HCQ. In another study, blood HCQ concentrations in HCQ-induced pigmentation cases were significantly higher than in controls (16). Thus, we surmise that higher HCQ doses may result in more frequent AEs.

This study had some limitations. The main limitation was the small number of patients who received low-dose HCQ. The study design was retrospective, and most rheumatologists chose the usual HCQ dose. In addition, blood HCQ concentrations were not measured in all patients, and we only compared usual-dose HCQ with low-dose HCQ. Although there was no significant difference in the renal function between the usual-dose and low-dose HCQ groups, patients could be assessed for treatment compliance only at consultation; differences in compliance may therefore have affected our results. Finally, our study had a short follow-up period of only six months. Immunological biomarkers in the low-dose HCQ group did not significantly improve over this period but may have improved over longer durations of observation. Since glucocorticoid therapy was typically reduced six months after starting HCQ administration owing to a lower disease activity, it was not possible to evaluate the effect of HCQ therapy beyond six months in this study. Furthermore, our study included some SLE patients with very mild disease activity, so our results may not be generalizable to clinical practice in other populations.

In conclusion, additional HCQ treatment is effective for SLE patients in the maintenance phase. Both doses of additional HCQ treatment were able to improve the skin involvement and composite measures of disease activity. The usual dose of HCQ may be more effective than a lower dose in reducing the immunological activity of SLE and may potentially lead to better outcomes. However, large trials are required to confirm the superiority of the usual dose of HCQ over low-dose HCQ.

The authors state that they have no Conflict of Interest (COI).

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