

Pharmacokinetic Aspects of Hydroxychloroquine and Its Relationship to Efficacy in Immunoglobulin A Nephropathy

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Keywords

IgA nephropathy · Hydroxychloroquine · Metabolite · Blood concentration · Efficacy

Abstract

Introduction: Hydroxychloroquine (HCQ) is recommended for Chinese patients with immunoglobulin A nephropathy (IgAN). This study aimed to investigate the pharmacokinetics of HCQ in the treatment of IgAN and its relationship with therapeutic efficacy. **Methods:** This prospective study included 49 IgAN patients treated with HCQ, who were divided into effective and ineffective groups based on HCQ treatment efficacy after 6 months, defined as a reduction in proteinuria of at least 50% from baseline. The concentrations of HCQ and its metabolites were measured by high-performance liquid chromatography-tandem mass spectrometry. The relationships between the concentrations of HCQ and its metabolites and therapeutic efficacy were analyzed using linear correlation analysis and logistic regression. Receiver operating characteristic (ROC) curves were generated to evaluate the predictive value of HCQ and its metabolite concentrations. **Results:** Following 6 months of treatment with HCQ, patients in the effective group exhibited increased concentrations of HCQ ($p = 0.022$) and

desethylchloroquine (DCQ) ($p = 0.015$). The results of the Spearman's correlation analysis indicated a positive correlation between alterations in proteinuria and concentrations of HCQ ($r = 0.328$, $p < 0.05$) and DCQ ($r = 0.267$, $p < 0.05$). Univariate and multivariate logistic regression analyses indicated that efficacy was significantly correlated with HCQ (odds ratio 1.008, 95% CI: 1.001–1.014) and DCQ (odds ratio 1.064, 95% CI: 1.010–1.121) concentrations. ROC curves indicated that an HCQ concentration of 442.6 ng/mL and a DCQ concentration of 42.7 ng/mL exhibited the optimal capacity to predict efficacy ($p < 0.05$). **Conclusion:** The blood concentrations of HCQ and its metabolite DCQ may be significant factors for evaluating therapeutic efficacy in IgAN patients.

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Introduction

Immunoglobulin A nephropathy (IgAN) is one of the most common types of glomerulonephritis and the leading cause of end-stage renal disease in Asian populations [1]. Proteinuria is considered the primary risk factor for the progression of IgAN to end-stage renal disease. Consequently, most treatments for IgAN aim to

reduce proteinuria [2, 3]. Previous studies have indicated that administering optimal supportive therapy with a renin-angiotensin-aldosterone system inhibitor (RAASi) is recommended for IgAN patients with proteinuria levels exceeding 0.5 g/day, regardless of their hypertensive status [4, 5]. The 2021 Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines suggest that patients at high risk of chronic kidney disease progression after 3 months of optimal therapy may be considered for corticosteroid (CS) or immunosuppressive therapy [6]. However, there is controversy and uncertainty regarding CS and immunosuppressive drugs efficacy in treating IgAN, as well as concerns about their adverse events (AEs) [7, 8]. Under these circumstances, effective and safe treatments are needed.

Hydroxychloroquine (HCQ) is a well-known immunomodulatory drug widely used to treat autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis [9, 10]. Several studies have shown that HCQ can effectively and safely reduce proteinuria in patients with IgAN. In a randomized controlled clinical trial (NCT02942381, AJKD), Liu et al. [11] reported that HCQ treatment significantly and safely reduced proteinuria in Chinese IgAN patients in addition to optimized RAASi treatment. Yang et al. [12] demonstrated that the antiproteinuric effect of HCQ is comparable to that of CS, but HCQ is much safer. The potential mechanism of action of HCQ in IgAN involves the interference with immune activation at various cellular levels, resulting in the suppression of both the innate and adaptive immune systems. In IgAN, mucosal toll-like receptor 9 (TLR-9) activation induces the overexpression of B-cell activating factor in dendritic cells, which stimulates the production of a proliferation-inducing ligand and interleukin-6 (IL-6), as well as promoting the production of galactose-deficient IgA1. HCQ interferes with TLR-9 ligand binding and TLR signaling via lysosomal inhibition, thereby inhibiting TLR-mediated cell activation and cytokine production [13]. Accordingly, the 2021 KDIGO guidelines propose that HCQ therapy may be employed for Chinese IgAN patients who do not achieve significant proteinuria remission despite optimized supportive therapy [6].

The clinical monitoring of drug concentrations is a common practice to ensure drug efficacy and minimize adverse effects. HCQ is absorbed in the upper intestinal tract and metabolized in the liver by cytochrome P450 (CYP450) enzymes. This process results in the formation of three primary metabolites: desethylhydroxychloroquine (DHCQ), desethylchloroquine (DCQ), and bisdesethylchloroquine (BDCQ) [14]. A correlation be-

tween blood concentrations of HCQ and its metabolites and clinical efficacy has been reported in patients with SLE. Geraldino-Pardilla and colleagues [15] demonstrated that a lower concentration of HCQ in the blood was an independent factor for greater disease activity in patients with SLE. In a recent study, Pan et al. [16] observed a negative association between the concentrations of HCQ and its metabolites and SLE disease activity after adjusting for potential confounding variables. However, no studies have assessed the value of HCQ and its metabolite blood concentrations in predicting the efficacy of HCQ in treating IgAN patients. To address these issues, we conducted this study.

Methods

Study Design and Population

Consecutive patients with IgAN were recruited from Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, for participation in this study. All subjects underwent renal puncture biopsy between January 2020 and June 2021. The diagnosis of IgAN was confirmed by immunofluorescence, while light microscopy and electron microscopy were utilized for detailed pathological evaluation, including the assessment of the MEST-C score. The study included patients with primary IgAN aged 18–75 years, with an estimated glomerular filtration rate (eGFR) >30 mL/min/1.73 m² (calculated using the chronic kidney disease Epidemiology Collaboration creatinine equation [17]) and no reduction in proteinuria from baseline after 3 months of optimal supportive therapy with RAASi. Patients with secondary IgAN, systemic use of CS or immunosuppressants within 3 months, planned pregnancy or breastfeeding, or contraindications to HCQ therapy were excluded. A total of 60 patients were enrolled in this study, all of whom were scheduled to be treated with HCQ (Fen-Le, SPH Zhongxi Pharmaceutical Co., Ltd., Shanghai, China) and optimal supportive therapy for at least 6 months. The administered HCQ dose varied according to the baseline eGFR. For patients with an eGFR >60 mL/min/1.73 m², 0.2 g HCQ was administered twice daily, and for those with 45 mL/min/1.73 m² $<$ eGFR <59 mL/min/1.73 m², 0.1 g HCQ was administered three times per day. Patients with 30 mL/min/1.73 m² $<$ eGFR <44 mL/min/1.73 m² were administered 0.1 g HCQ twice daily [11]. The study was terminated if patients experienced serious AEs or a decrease in eGFR $>30\%$ during the trial. A total of 49 patients completed the trial and were divided into two groups based on whether their

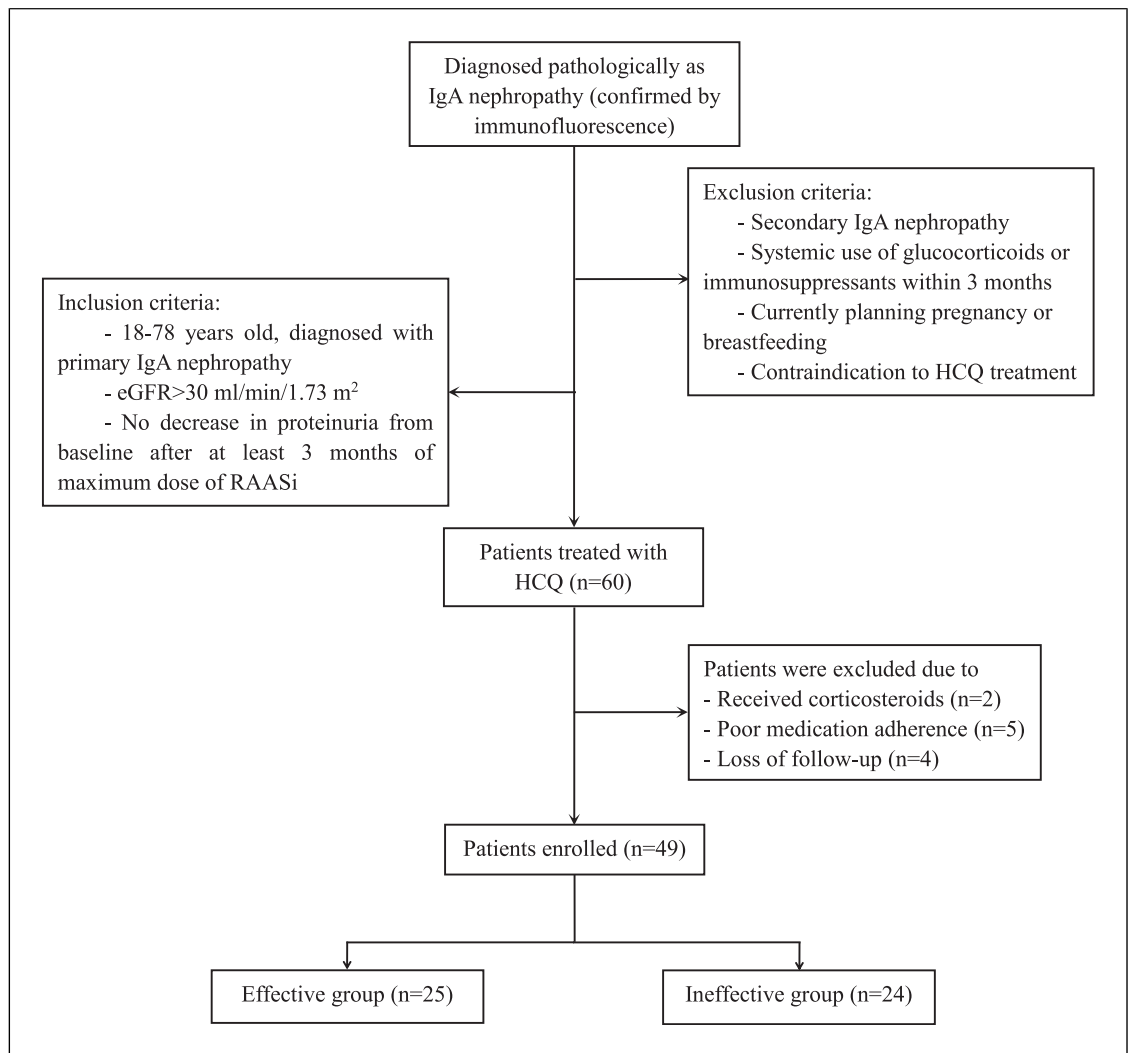


Fig. 1. Study flowchart.

final proteinuria decreased by >50% from baseline. The flowchart is shown in Figure 1.

This prospective clinical trial was reviewed and approved by the Medical Ethics Committee of Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University (approval No. 2021-074-05), and registered with the Chinese Clinical Trial Registry (Registration No.: ChiCTR2100052910). All participants provided written informed consent before their enrollment in the study.

Data Acquisition

The following data were recorded: patient demographics (age, sex, body mass index [BMI], blood pressure), laboratory parameters related to HCQ pharmacokinetics (liver

and kidney function, albumin levels), use of RAASi, daily dosage and duration of HCQ therapy, AEs related to HCQ administration, blood concentrations of HCQ and its metabolites, and pathological data (MEST-C score according to the Oxford classification of IgAN [18]).

Determination of Blood Concentrations of HCQ and Its Metabolites

Blood samples were collected prior to the administration of HCQ while patients were fasting to ensure consistent measurements. Samples were obtained in the morning between 8:00 a.m. and 10:00 a.m. to minimize variability due to diurnal fluctuations. Peripheral blood samples were collected using EDTA anticoagulation tubes, and the concentrations of HCQ, DHCQ, DCQ, and

BDCQ were subsequently measured. The measured blood concentrations of HCQ and its metabolites were trough concentrations, defined as the lowest concentration of the drug in the blood, collected before the administration of the next dose of HCQ. HPLC-MS/MS was used in this study to determine the concentrations of HCQ and its metabolites. The standard products were HCQ (H0915, Sigma-Aldrich, USA), DHCQ (17-MJC-106-5, Toronto Research Chemicals, Canada), DCQ (ZZS18030101, Shanghai Zhenzhun Biotechnology Co., Ltd., China), and BDCQ (SMB00975, Sigma-Aldrich, USA). The internal standard was chloroquine (C1650000, Sigma-Aldrich, USA). An Agilent 1200 Series HPLC system was used. Method validation involved determining the linear range, accuracy, precision, and limits of quantification. The detailed procedures and the acceptance criteria used to validate the assay have been described previously [19].

Statistical Analysis

Continuous data are described as the means $\pm$ standard deviations if normally distributed or as medians with interquartile ranges (IQRs) if not normally distributed. Categorical variables are described as frequencies and percentages. Differences between the two groups were tested using the independent-samples *t* test or Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. To compare multiple data groups, one-way ANOVA with post hoc multiple comparisons was used; for nonnormally distributed data, the nonparametric Kruskal-Wallis test was used. Pearson's test or, where appropriate, Spearman's test was used for correlation analysis. Univariate and multivariate logistic regression analyses were conducted to assess the associations between the blood concentrations of HCQ and its metabolites and therapeutic efficacy. The Hosmer-Lemeshow test was utilized to evaluate the validity of the regression model. Receiver operating characteristic (ROC) curves were used to evaluate the predictive value of HCQ and its metabolite blood concentrations. The data were analyzed using SPSS 26.0 software (SPSS, Chicago, IL, USA) and GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, CA, USA). *p* values <0.05 were considered to indicate statistical significance.

Results

Demographic Characteristics and Clinical Data

A total of 49 patients completed the full study and were divided into two groups based on whether their proteinuria decreased by more than 50% from baseline. The

characteristics of both groups are shown in Table 1. The baseline age, sex ratio, BMI, histopathological score, and laboratory indices (proteinuria, eGFR, liver function, lipid indices, etc.) were similar between the effective and ineffective groups. Patients in the effective group exhibited lower proteinuria after 6 months of treatment than did those in the ineffective group (329 mg/d [IQR: 227–501 mg/d] vs. 765 mg/d [IQR: 540–1,288 mg/d]; *p* = 0.001). Furthermore, patients in the effective group had higher blood concentrations of HCQ (476.8 ng/mL [IQR: 346.1–551.7 ng/mL] vs. 323.8 ng/mL [IQR: 164.9–440.0 ng/mL]; *p* = 0.022) and DCQ (49.11 ng/mL [IQR: 38.56–56.72 ng/mL] vs. 28.31 ng/mL [IQR: 11.51–56.20 ng/mL]; *p* = 0.015) than did those in the ineffective group, whereas there was no significant difference in DHCQ (285.3 ng/mL [IQR: 208.4–393.5 ng/mL] vs. 205.8 ng/mL [IQR: 132.8–375.0 ng/mL]; *p* = 0.108) or BDCQ (39.12 ng/mL [IQR: 19.09–59.85 ng/mL] vs. 24.55 ng/mL [IQR: 12.70–44.60 ng/mL]; *p* = 0.187) blood concentrations between the two groups.

Correlation of the Blood Concentrations of HCQ and Its Metabolites with HCQ Efficacy

Spearman correlation analysis was used to explore the degree of association between the blood concentrations of HCQ and its metabolites and the decreased rate of proteinuria in IgAN patients. Our results showed that the decreased rate of proteinuria was significantly and positively correlated with the blood concentrations of HCQ (*r* = 0.328, *p* = 0.021) (Fig. 2a) and DCQ (*r* = 0.267, *p* = 0.048) (Fig. 2d) but not with those of DHCQ (*r* = 0.206, *p* = 0.156) (Fig. 2b) or BDCQ (*r* = 0.196, *p* = 0.177) (Fig. 2c).

The efficacy of HCQ was evaluated as a binary outcome variable in IgAN patients. Univariate logistic regression analyses demonstrated that HCQ efficacy was associated with blood concentrations of HCQ (OR: 1.004; 95% CI: 1.000–1.007; *p* = 0.029) and DCQ (OR: 1.032; 95% CI: 1.004–1.061; *p* = 0.025). According to the multivariate regression model, the efficacy of HCQ remained significantly associated with the blood concentrations of HCQ (OR: 1.008; 95% CI: 1.001–1.014; *p* = 0.021) and DCQ (OR: 1.064; 95% CI: 1.010–1.121; *p* = 0.021) after adjusting for age, sex, BMI, baseline proteinuria, baseline eGFR, DHCQ concentration, and BDCQ concentration (Table 2).

ROC Curves of Blood Concentrations of HCQ and Its Metabolites

Based on the logistic regression analysis, we additionally generated ROC curves for HCQ and its metabolite blood concentrations to predict HCQ efficacy

Table 1. Characteristics of patients in the effective and ineffective groups

Variables	Total (n = 49)	Effective group (n = 25)	Ineffective group (n = 24)	p value
Sex: male (%)	24 (49)	13 (52)	11 (46)	0.667
Age, years	36 (29, 47)	34 (26, 47)	36 (32, 47)	0.531
Weight, kg	65.8±12.4	65.5±10.1	66.1±14.6	0.873
BMI, kg/m ²	24.1±3.4	24.1±3.0	24.2±3.9	0.924
SBP, mm Hg	129.0±17.0	128.1±14.0	129.9±20.4	0.726
DBP, mm Hg	90.0±12.0	90.3±10.6	89.7±13.5	0.869
ALT, U/L	15 (11, 20)	14 (11, 18)	17 (12, 25)	0.286
AST, U/L	16.7 (15.0, 20.0)	16.3 (14.6, 18.8)	17.4 (15.2, 22.7)	0.211
ALB, g/L	39.5±3.5	39.4±3.2	39.6±3.7	0.814
Cr, µmol/L	78.0±25.9	76.0±26.1	79.8±26.1	0.625
eGFR at baseline, mL/min/1.73 m ²	105.0±15.0	106.7±15.9	103.3±14	0.427
eGFR at 6 months, mL/min/1.73 m ²	103.7±13.9	105.5±14.3	101.8±13.5	0.365
Oxford histologic score				
M 0/1	1/48	0/25	1/23	0.490
E 0/1	49/0	25/0	24/0	1
S 0/1	32/17	14/11	18/6	0.162
T 0/1/2	40/6/3	21/3/1	19/3/2	0.869
C 0/1/2	31/16/2	14/10/1	17/6/1	0.673
Therapy with RAASi				
ACEI alone	4	1	3	
ARB alone	45	24	21	
Proteinuria at baseline, mg/d	921 (676, 1,580)	1,073 (766, 1,998)	859 (492, 1,361)	0.468
Proteinuria at 6 months, mg/d	514 (287, 861)	329 (227, 501)	765 (540, 1,288)	0.001
HCQ concentration, ng/mL	435.4 (234.3, 506.8)	476.8 (346.1, 551.7)	323.8 (164.9, 440.0)	0.022
DHCQ concentration, ng/mL	260.6 (159.7, 388.2)	285.3 (208.4, 393.5)	205.8 (132.8, 375.0)	0.108
BDCQ concentration, ng/mL	31.84 (14.68, 49.09)	39.12 (19.09, 59.85)	24.55 (12.70, 44.60)	0.187
DCQ concentration, ng/mL	43.12 (21.70, 56.72)	49.11 (38.56, 56.72)	28.31 (11.51, 56.20)	0.015

Significant *p* values are in bold. The concentrations of HCQ, DHCQ, BDCQ, and DCQ correspond to serum levels. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine transaminase; AST, aspartate transaminase; ALB, albumin; Cr, creatinine; eGFR, estimated glomerular filtration rate; RAASi, renin-angiotensin-aldosterone system; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; M, mesangial hypercellularity (M0, <50% of glomeruli show mesangial hypercellularity; M1, >50% of glomeruli show mesangial hypercellularity); E, endocapillary hypercellularity (E0, no endocapillary hypercellularity; E1, any glomeruli show endocapillary hypercellularity); S, segmental glomerulosclerosis (S0, absent; S1, present in any glomeruli); T, tubular atrophy/interstitial fibrosis (T0, 0%–25% of cortical area; T1, 26%–50% of cortical area; T2, >50% of cortical area); C, crescents (C0, absent; C1, 0%–25% of glomeruli; C2, ≥25% of glomeruli); HCQ, hydroxychloroquine; DHCQ, desethylhydroxychloroquine; BDCQ, bisdesethylchloroquine; DCQ, desethylchloroquine.

(Fig. 3). The area under the ROC curve (AUC) was used to indicate the accuracy of HCQ and its metabolite blood concentrations in predicting the efficacy of HCQ. The AUC for the HCQ blood concentration was 0.72, whereas the AUC for the DCQ blood concentration was 0.70. Furthermore, the AUC for HCQ combined with blood concentrations of HCQ and DCQ for the pre-

diction of HCQ efficacy was 0.71. There was no significant difference in the AUC values of the HCQ blood concentration, DCQ blood concentration, or their combination (*p* > 0.05) (Table 3). The cutoff values for the blood concentrations of HCQ and DCQ were 442.6 ng/mL and 42.7 ng/mL, respectively. Based on these cutoff values, patients were classified into high

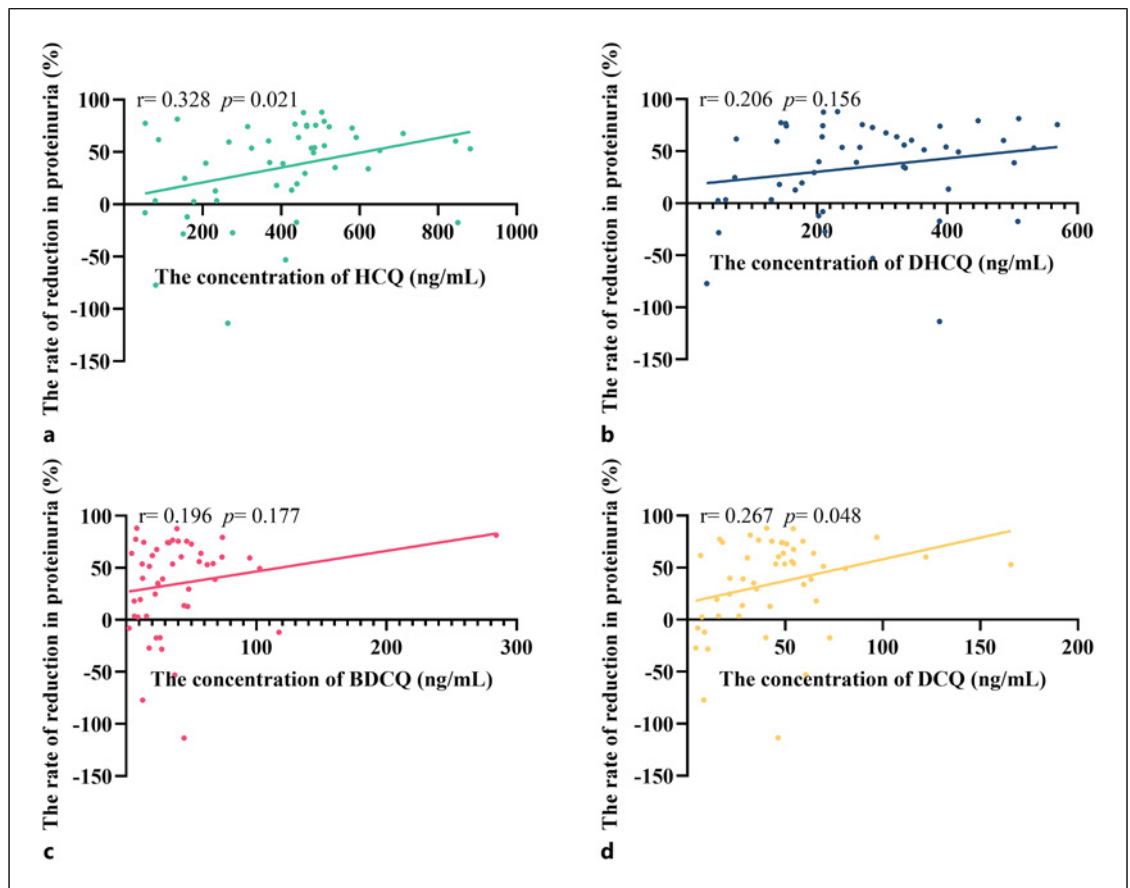


Fig. 2. Correlation analysis of the blood concentrations of HCQ and its metabolites with the rate of reduction in proteinuria. **a** The blood concentration of HCQ was positively correlated with the rate of reduction in proteinuria ($r = 0.328$, $p = 0.021$). **b** There was no correlation between the DHCQ blood concentration and the rate of reduction in proteinuria ($r = 0.206$, $p = 0.156$). **c** There was no

correlation between the BDCQ blood concentration and the rate of reduction in proteinuria ($r = 0.196$, $p = 0.177$). **d** The DCQ blood concentration was positively correlated with the rate of reduction in proteinuria ($r = 0.267$, $p = 0.048$). HCQ, hydroxychloroquine; DHCQ, desethylhydroxychloroquine; BDCQ, bisdesethylchloroquine; DCQ, desethylchloroquine.

Table 2. Univariate and multivariate regression models evaluating the association between HCQ and DCQ blood concentrations and HCQ efficacy

Variable	OR (95% CI)		p value	
	crude	adjusted ¹	crude	adjusted ¹
HCQ concentration	1.004 (1.000, 1.007)	1.008 (1.001, 1.014)	0.029	0.021
DCQ concentration	1.032 (1.004, 1.061)	1.064 (1.010, 1.121)	0.025	0.021

BMI, body mass index; eGFR, estimated glomerular filtration rate; HCQ, hydroxychloroquine; DHCQ, desethylhydroxychloroquine; BDCQ, bisdesethylchloroquine; DCQ, desethylchloroquine. ¹Adjusted for age, sex, BMI, proteinuria at baseline, eGFR at baseline, DHCQ concentration, BDCQ concentration.

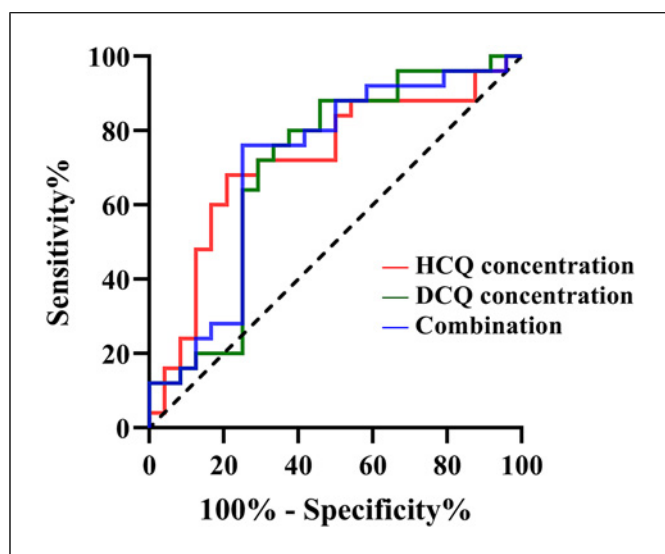


Fig. 3. ROC curves of HCQ blood concentrations, DCQ blood concentrations, and their combination for predicting HCQ efficacy. HCQ, hydroxychloroquine; DCQ, desethylchloroquine.

and low HCQ blood concentration groups and high and low DCQ blood concentration groups. The results demonstrated a statistically significant reduction in proteinuria following HCQ treatment in patients with high concentrations of HCQ (1173 mg/d [IQR: 721–2236 mg/d] vs. 446 mg/d [IQR: 237–935 mg/d]; $p = 0.001$) (Fig. 4a) and DCQ (965 mg/d [IQR: 721–2023 mg/d] vs. 427 mg/d [IQR: 253–1095 mg/d]; $p = 0.004$) (Fig. 4c). In contrast, there was no discernible change in proteinuria levels following HCQ treatment in patients with low HCQ (859 mg/d [IQR: 492–1315 mg/d] vs. 567 mg/d [IQR: 420–844 mg/d]; $p = 0.222$) (Fig. 4b) or DCQ (919 mg/d [IQR: 492–1482 mg/d] vs. 641 mg/d [IQR: 424–837 mg/d]; $p = 0.074$) (Fig. 4d) blood concentrations.

Safety and AEs

No serious AEs were reported during this study. Two patients were transferred to alternative therapeutic regimens due to nausea. None of the patients were admitted to the hospital for AEs.

Discussion

RAASi therapy is the recommended foundational supportive treatment for IgAN [20]. However, previous studies have indicated that some patients with high baseline levels of proteinuria may not respond well to

this treatment [21]. HCQ is an additional therapy used in combination with RAASi, and it has been shown to have favorable and safe antiproteinuric effects in patients with IgAN [11, 12]. Nonetheless, the relationship between the blood concentrations of HCQ and its metabolites and the efficacy of HCQ in the treatment of IgAN patients has not yet been elucidated. Our study revealed that the blood concentrations of HCQ and its metabolite DCQ may be significant factors in evaluating the therapeutic efficacy of HCQ in patients with IgAN.

Monitoring blood concentrations of drugs is a common practice in clinical settings to ensure their efficacy and reduce adverse effects [22]. Blood concentrations of HCQ have been shown to correlate with clinical outcomes. Costedoat-Chalumeau et al. [23] suggested that low HCQ blood concentrations are a marker and predictor of disease progression in patients with SLE. Furthermore, Liu et al. [22] proposed that HCQ blood concentrations may be crucial in evaluating treatment efficacy in patients with SLE. This finding aligns with our findings in patients with IgAN. In this study, we observed that IgAN patients undergoing effective HCQ treatment exhibited elevated blood concentrations of HCQ and its metabolite DCQ. HCQ and DCQ blood concentrations are independent factors influencing HCQ efficacy. Additionally, we found that the rate of proteinuria reduction in IgAN patients was positively correlated with the blood concentrations of HCQ and DCQ.

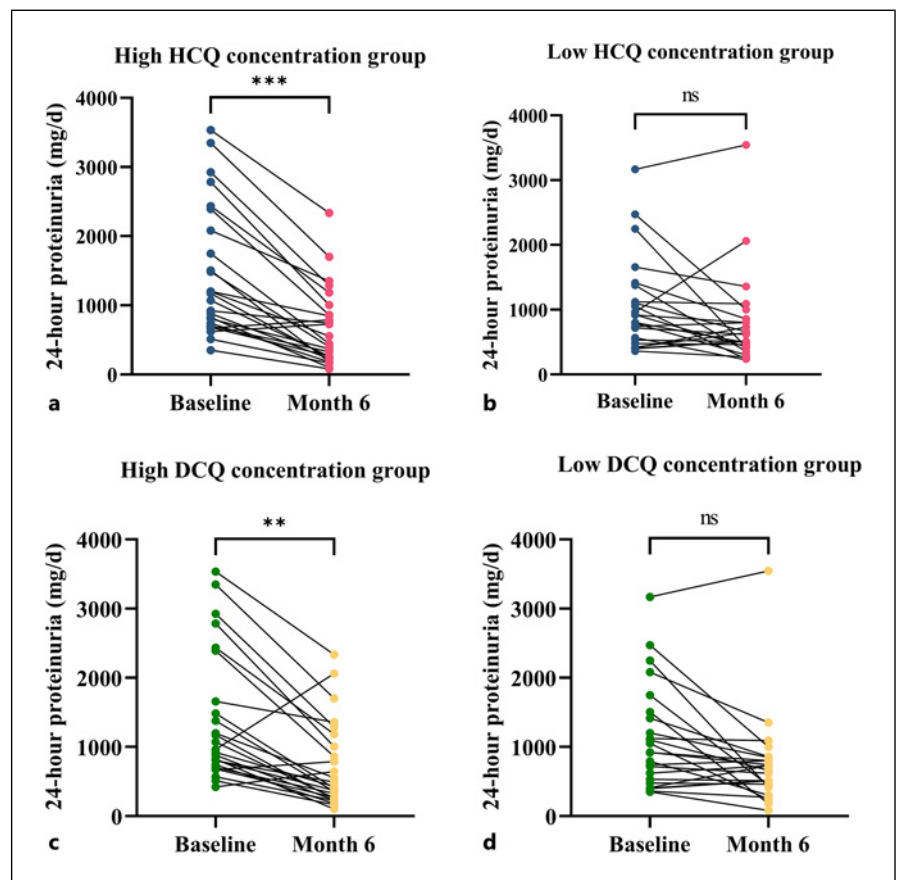
Although several studies have demonstrated a correlation between the blood concentration of HCQ and treatment efficacy, the specific effective blood concentration of HCQ remains undefined. In patients with lupus nephritis, blood concentrations of HCQ less than 613.5 ng/mL have been reported to predict flares [24]. In contrast, an earlier study by Costedoat-Chalumeau et al. [23] concluded that the effective HCQ concentration was 1,000 ng/mL in patients with SLE. However, a subsequent study, which achieved the target blood concentration by adjusting the HCQ dose, did not demonstrate a significant reduction in flares compared to patients who did not have the HCQ dose adjusted. Consequently, the effective blood concentration of HCQ remains a matter of contention [25]. Interestingly, a study by Durcan et al. [26] sought to clarify this controversy by proposing that blood concentrations of HCQ greater than 500 ng/mL in patients with SLE may serve as a threshold for drug effectiveness. In our study, we plotted ROC curves for HCQ treatment, identifying a cutoff value of 442.6 ng/mL for the blood

Table 3. ROC analysis results and pairwise comparison of the AUC among the three models

	AUC (95% CI)	Cutoff value	<i>p</i> value	Pairwise ROC curve comparison	<i>p</i> value
Model 1	0.72 (0.57–0.87)	442.6	0.008	Model 1 versus Model 2	0.804
Model 2	0.70 (0.55–0.86)	42.7	0.015	Model 1 versus Model 3	0.859
Model 3	0.71 (0.56–0.86)	–	0.012	Model 2 versus Model 3	0.687

Model 1, ROC curve of HCQ concentration; Model 2, ROC curve of DCQ concentration; Model 3, ROC curve of HCQ concentration combined with DCQ concentration. ROC, receiver operating characteristic; AUC, area under the curve; HCQ, hydroxychloroquine; DCQ, desethylchloroquine.

Fig. 4. Changes in proteinuria before and after HCQ treatment in IgAN patients with different HCQ and DCQ blood concentration groups. **a** Patients in the high-HCQ blood concentration group had significantly lower proteinuria after 6 months of HCQ therapy ($p = 0.001$). **b** Patients in the low HCQ blood concentration group showed no significant change in proteinuria after 6 months of HCQ treatment ($p = 0.222$). **c** Patients in the high-DCQ blood concentration group had significantly lower proteinuria after 6 months of HCQ therapy ($p = 0.004$). **d** Patients in the low DCQ blood concentration group showed no significant change in proteinuria after 6 months of HCQ treatment ($p = 0.074$). ** $p < 0.01$, *** $p < 0.001$. HCQ, hydroxychloroquine; DCQ, desethylchloroquine.



concentration of HCQ and 42.7 ng/mL for DCQ. These findings indicate that HCQ exhibits varying therapeutic thresholds across a range of immune-mediated disorders. In the context of IgAN, the HCQ threshold is comparatively lower than that observed in SLE, which may reflect differing mechanisms of action of HCQ across these clinical presentations. Nevertheless, it is essential to consider inter-individual variability and therapeutic response in each patient. Accordingly, in

clinical practice, it is more scientific and reasonable to adjust HCQ dosage by monitoring the concentrations of HCQ and its metabolites for different diseases. Grouping patients according to these cutoff values revealed a significant reduction in proteinuria in the group with high HCQ and DCQ concentrations, while no substantial change in proteinuria was observed in the group with low HCQ and DCQ concentrations. Notably, there were no significant differences in the

AUC values for HCQ and DCQ blood concentrations, nor for their combination. This suggests that HCQ and DCQ possess similar predictive value and that their combination does not demonstrate enhanced predictive power. Thus, we believe that monitoring the concentrations of either HCQ or DCQ may be sufficient to assess efficacy. Nevertheless, further studies are recommended to determine whether monitoring these two metabolites provides greater insights into treatment responses and potential adverse effects and to establish clear guidelines for monitoring methods in patients with IgAN.

HCQ is believed to exert a range of immunomodulatory effects, including the inhibition of inflammatory cell activation, autoantigen presentation, TLR signaling, and cytokine or chemokine production [27, 28]. The mechanism by which HCQ reduces proteinuria in patients with IgAN may involve inhibiting mucosal and intrarenal TLR signaling pathways, which attenuate intrarenal inflammation and reduce galactose-deficient IgA1 synthesis [29, 30]. Following oral administration, HCQ is rapidly absorbed from the gastrointestinal tract into the bloodstream, where it reaches its maximum concentration within 3–6 h [31]. When HCQ is administered at a consistent dose for at least 6 months, the concentrations of HCQ and its metabolites in the blood may reach a stable equilibrium [32]. Consequently, we selected the blood concentrations at 6 months after HCQ treatment to assess its efficacy. HCQ is metabolized in the liver by demethylation to DHCQ, DCQ, and BDCQ [33]. The reduced groups on the side chains of DHCQ, DCQ, and BDCQ differ. However, their primary chemical structures remain identical to those of HCQ. This may explain why they exhibit similar immunomodulatory effects to those of HCQ. Previous studies have generally concluded that there is a correlation between HCQ blood concentrations and therapeutic efficacy [22, 23, 26], while the relationship between HCQ metabolite blood concentrations and therapeutic efficacy remains controversial. Durcan et al. [26] and Yeon Lee et al. [34] reported a negative correlation between DHCQ blood concentrations and SLE disease activity. Similarly, Pan et al. [16] suggested that DHCQ and BDCQ exhibited a negative correlation with SLE disease activity. However, a recent study failed to identify a correlation between DHCQ, BDCQ, and DCQ blood concentrations and SLE disease activity [35]. To our knowledge, we are the first to investigate the relationship between blood concentrations of HCQ metabolites and treatment efficacy in patients with IgAN. The results

demonstrated a positive correlation between DCQ blood concentrations and a reduction in proteinuria in IgAN patients.

The current dose of HCQ administered to patients with IgAN is primarily based on the eGFR, with a maximum daily dose of no more than 400 mg [11, 12]. However, HCQ exhibits considerable pharmacokinetic variability, with blood concentrations varying significantly between patients, even when they receive the same dose. This interindividual difference is not fully understood. Pharmacogenetics polymorphisms can affect the rate of HCQ metabolism *in vivo* and may be an important reason for differences in blood concentrations [34]. HCQ is metabolized by CYP450 enzymes (CYP2D6, CYP2C8, CYP3A4, and CYP3A5) into three products: DHCQ, DCQ, and BDCQ. Single nucleotide polymorphisms in CYP450 enzymes may be important determinants of differences in blood concentrations of HCQ among patients. A recent study involving 489 Chinese patients with SLE revealed that the TC and CC genotypes of CYP2C8 (rs7910936) were significantly associated with increased blood concentrations of HCQ. In contrast, the TT genotype of CYP2C8 (rs10882521) was associated with decreased blood concentrations of HCQ. Furthermore, polymorphisms in CYP2D6*10 (rs1065852) influence the equilibrium of HCQ with its metabolite DHCQ [36]. No studies have investigated the relationships between genetic polymorphisms and blood concentrations of HCQ and its metabolites in patients with IgAN. It is postulated that polymorphisms in CYP450 enzymes are similarly associated with blood concentrations of HCQ and its metabolites in IgAN patients. Therefore, genotypic testing and regular monitoring of blood levels in IgAN patients could be beneficial for adjusting the dosage of HCQ administered.

It should be noted that the present study is subject to certain limitations. First, the study was conducted at a single center and had a relatively small sample size. Furthermore, the limited observation period precluded the investigation of the relationship between blood concentrations of HCQ and its metabolites and long-term prognosis and drug safety. Consequently, further studies with larger sample sizes and longer observation periods are essential to validate and confirm these results.

In conclusion, the results of this study indicate that the blood concentrations of HCQ and its metabolite DCQ are positively correlated with a reduction in proteinuria. This finding suggested that HCQ and its metabolite DCQ may play a role in the immunomodulatory treatment of IgAN.

Furthermore, ROC curve analysis revealed that blood concentrations of HCQ above 442.6 ng/mL and blood concentrations of DCQ above 42.7 ng/mL might be effective concentrations for the efficacy of HCQ. Consequently, monitoring the blood concentrations of HCQ and its metabolite DCQ in IgAN patients may prove beneficial for the clinical management of these patients.

Statement of Ethics

This study was reviewed and approved by the Medical Ethics Committee of the Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University (approval No. 2021-074-05). All participants provided written informed consent before their enrollment in the study.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

References

- Rodrigues JC, Haas M, Reich HN. IgA nephropathy. *Clin J Am Soc Nephrol*. 2017; 12(4):677–86. <https://doi.org/10.2215/CJN.07420716>
- Le W, Liang S, Hu Y, Deng K, Bao H, Zeng C, et al. Long-term renal survival and related risk factors in patients with IgA nephropathy: results from a cohort of 1155 cases in a Chinese adult population. *Nephrol Dial Transplant*. 2012;27(4):1479–85. <https://doi.org/10.1093/ndt/gfr527>
- Goto M, Wakai K, Kawamura T, Ando M, Endoh M, Tomino Y. A scoring system to predict renal outcome in IgA nephropathy: a nationwide 10-year prospective cohort study. *Nephrol Dial Transplant*. 2009;24(10): 3068–74. <https://doi.org/10.1093/ndt/gfp273>
- Floege J, Rauen T, Tang SCW. Current treatment of IgA nephropathy. *Semin Immunopathol*. 2021;43(5):717–28. <https://doi.org/10.1007/s00281-021-00888-3>
- Maixnerova D, Tesar V. Emerging modes of treatment of IgA nephropathy. *Int J Mol Sci*. 2020;21(23):9064. <https://doi.org/10.3390/ijms21239064>
- Kidney Disease: Improving Global Outcomes KDIGO Glomerular Diseases Work Group. Kidney disease: improving global outcomes glomerular diseases work G: KDIGO 2021 clinical practice guideline for the management of glomerular diseases. *Kidney Int*. 2021;100(4S):S1–276. <https://doi.org/10.1016/j.kint.2021.05.021>
- Rauen T, Eitner F, Fitzner C, Sommerer C, Zeier M, Otte B, et al. Intensive supportive care plus immunosuppression in IgA nephropathy. *N Engl J Med*. 2015; 373(23):2225–36. <https://doi.org/10.1056/NEJMoa1415463>
- Rauen T, Wied S, Fitzner C, Eitner F, Sommerer C, Zeier M, et al. After ten years of follow-up, no difference between supportive care plus immunosuppression and supportive care alone in IgA nephropathy. *Kidney Int*. 2020;98(4):1044–52. <https://doi.org/10.1016/j.kint.2020.04.046>
- Olsen NJ, Schleich MA, Karp DR. Multifaceted effects of hydroxychloroquine in human disease. *Semin Arthritis Rheum*. 2013;43(2):264–72. <https://doi.org/10.1016/j.semarthrit.2013.01.001>
- Rempennault C, Combe B, Barnette T, Gaujoux-Viala C, Lukas C, Morel J, et al. Metabolic and cardiovascular benefits of hydroxychloroquine in patients with rheumatoid arthritis: a systematic review and meta-analysis. *Ann Rheum Dis*. 2018;77(1): 98–103. <https://doi.org/10.1136/annrheumdis-2017-211836>
- Liu LJ, Yang YZ, Shi SF, Bao YF, Yang C, Zhu SN, et al. Effects of hydroxychloroquine on proteinuria in IgA nephropathy: a randomized controlled trial. *Am J Kidney Dis*. 2019; 74(1):15–22. <https://doi.org/10.1053/j.ajkd.2019.01.026>
- Yang YZ, Chen P, Liu LJ, Cai QQ, Shi SF, Chen YQ, et al. Comparison of the effects of hydroxychloroquine and corticosteroid treatment on proteinuria in IgA nephropathy: a case-control study. *BMC Nephrol*. 2019;20(1):297. <https://doi.org/10.1186/s12882-019-1488-6>
- Stefan G, Mircescu G. Hydroxychloroquine in IgA nephropathy: a systematic review. *Ren Fail*. 2021;43(1):1520–7. <https://doi.org/10.1080/0886022X.2021.2000875>
- Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat Rev Rheumatol*. 2020;16(3): 155–66. <https://doi.org/10.1038/s41584-020-0372-x>
- Geraldino-Pardilla L, Perel-Winkler A, Miceli J, Neville K, Danias G, Nguyen S, et al. Association between hydroxychloroquine levels and disease activity in a predominantly Hispanic systemic lupus erythematosus cohort. *Lupus*. 2019;28(7):862–7. <https://doi.org/10.1177/0961203319851558>
- Pan M, Jin R, Dai Y, Gao B, Liu Y, Peng X, et al. The presumable effects of hydroxychloroquine and its metabolites in the treatment of systemic lupus erythematosus. *Int Immunopharmacol*. 2024;126:111269. <https://doi.org/10.1016/j.intimp.2023.111269>
- Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012; 367(1):20–9. <https://doi.org/10.1056/NEJMoa1114248>
- Trimarchi H, Barratt J, Catran DC, Cook HT, Coppo R, Haas M, et al. Oxford classification of IgA nephropathy 2016: an update from the IgA nephropathy classification working group. *Kidney Int*. 2017;91(5): 1014–21. <https://doi.org/10.1016/j.kint.2017.02.003>

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Author Contributions

Yaotong Shi, Ting Yang, and Ye Wang were responsible for the conception and design of the study. Yaotong Shi drafted the manuscript. Yaotong Shi, Nan Li, and Qiuyuan Shao were responsible for data acquisition and analysis. Chunming Jiang provided the patients and participated in the manuscript revision. Jing Liu read and approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

- 19 Soichot M, Megarbane B, Houze P, Chevallard L, Fonsart J, Baud FJ, et al. Development, validation and clinical application of a LC-MS/MS method for the simultaneous quantification of hydroxychloroquine and its active metabolites in human whole blood. *J Pharm Biomed Anal.* 2014;100:131–7. <https://doi.org/10.1016/j.jpba.2014.07.009>
- 20 Obrisca B, Sinescu I, Ismail G, Mircescu G. Has the time arrived to refine the indications of immunosuppressive therapy and prognosis in IgA nephropathy? *J Clin Med.* 2019;8(10):1584. <https://doi.org/10.3390/jcm8101584>
- 21 Li PK, Leung CB, Chow KM, Cheng YL, Fung SK, Mak SK, et al. Hong Kong study using valsartan in IgA nephropathy (HKVIN): a double-blind, randomized, placebo-controlled study. *Am J Kidney Dis.* 2006;47(5):751–60. <https://doi.org/10.1053/j.ajkd.2006.01.017>
- 22 Liu PC, Luo SL, Lv MN, Wang Y, Li JB, Yu SJ, et al. Effect of hydroxychloroquine blood concentration on the efficacy and ocular toxicity of systemic lupus erythematosus. *Sci Rep.* 2024;14(1):7674. <https://doi.org/10.1038/s41598-024-58391-2>
- 23 Costedoat-Chalumeau N, Amoura Z, Hulot JS, Hammoud HA, Aymard G, Cacoub P, et al. Low blood concentration of hydroxychloroquine is a marker for and predictor of disease exacerbations in patients with systemic lupus erythematosus. *Arthritis Rheum.* 2006;54(10):3284–90. <https://doi.org/10.1002/art.22156>
- 24 Pedrosa TN, Pasoto SG, Aikawa NE, Yuki EF, Borba EF, Filho JCF, et al. Understanding the dynamics of hydroxychloroquine blood levels in lupus nephritis. *Lupus.* 2020;29(6):560–8. <https://doi.org/10.1177/0961203320912832>
- 25 Costedoat-Chalumeau N, Galicier L, Aumaitre O, Frances C, Le Guern V, Liote F, et al. Hydroxychloroquine in systemic lupus erythematosus: results of a French multicentre controlled trial (PLUS Study). *Ann Rheum Dis.* 2013;72(11):1786–92. <https://doi.org/10.1136/annrheumdis-2012-202322>
- 26 Durcan L, Clarke WA, Magder LS, Petri M. Hydroxychloroquine blood levels in systemic lupus erythematosus: clarifying dosing controversies and improving adherence. *J Rheumatol.* 2015;42(11):2092–7. <https://doi.org/10.3899/jrheum.150379>
- 27 Kuznik A, Bencina M, Svajger U, Jeras M, Rozman B, Jerala R. Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. *J Immunol.* 2011;186(8):4794–804. <https://doi.org/10.4049/jimmunol.1000702>
- 28 Goldman FD, Gilman AL, Hollenback C, Kato RM, Premack BA, Rawlings DJ. Hydroxychloroquine inhibits calcium signals in T cells: a new mechanism to explain its immunomodulatory properties. *Blood.* 2000;95(11):3460–6. <https://doi.org/10.1182/blood.V95.11.3460>
- 29 Barratt J, Eitner F, Feehally J, Floege J. Immune complex formation in IgA nephropathy: a case of the ‘right’ antibodies in the ‘wrong’ place at the ‘wrong’ time? *Nephrol Dial Transplant.* 2009;24(12):3620–3. <https://doi.org/10.1093/ndt/gfp441>
- 30 Makita Y, Suzuki H, Kano T, Takahata A, Julian BA, Novak J, et al. TLR9 activation induces aberrant IgA glycosylation via APRIL- and IL-6-mediated pathways in IgA nephropathy. *Kidney Int.* 2020;97(2):340–9. <https://doi.org/10.1016/j.kint.2019.08.022>
- 31 Tett SE, Cutler DJ, Day RO. Bioavailability of hydroxychloroquine tablets assessed with deconvolution techniques. *J Pharm Sci.* 1992;81(2):155–9. <https://doi.org/10.1002/jps.2600810211>
- 32 Tett SE, Cutler DJ, Day RO, Brown KF. Bioavailability of hydroxychloroquine tablets in healthy volunteers. *Br J Clin Pharmacol.* 1989;27(6):771–9. <https://doi.org/10.1111/j.1365-2125.1989.tb03439.x>
- 33 Richard SA, Kampo S, Hechavarria ME, Sackey M, Buunaaim ADB, Kuugbee ED, et al. Elucidating the pivotal immunomodulatory and anti-inflammatory potentials of chloroquine and hydroxychloroquine. *J Immunol Res.* 2020;2020:4582612. <https://doi.org/10.1155/2020/4582612>
- 34 Yeon Lee J, Lee J, Ki Kwok S, Hyeon Ju J, Su Park K, Park SH. Factors related to blood hydroxychloroquine concentration in patients with systemic lupus erythematosus. *Arthritis Care Res.* 2017;69(4):536–42. <https://doi.org/10.1002/acr.22962>
- 35 Eryavuz Onmaz D, Tezcan D, Abusoglu S, Yilmaz S, Yerlikaya FH, Onmaz M, et al. Effects of hydroxychloroquine and its metabolites in patients with connective tissue diseases. *Inflammopharmacology.* 2021;29(6):1795–805. <https://doi.org/10.1007/s10787-021-00887-8>
- 36 Xie H, Wen X, Wang Y, Huang X, Shu Q, Wang D, et al. Genotype-guided new approach for dose optimisation of hydroxychloroquine administration in Chinese patients with SLE. *Lupus Sci Med.* 2023;10(2):e000997. <https://doi.org/10.1136/lupus-2023-000997>