Hydroxychloroquine Use Is Associated with Lower Odds of Persistently Positive Antiphospholipid Antibodies and/or Lupus Anticoagulant in Systemic Lupus Erythematosus

ANNA BRODER and CHAIM PUTTERMAN

ABSTRACT. ***Objective.*** Antiphospholipid antibodies (aPL) play an active role in the pathogenesis of the antiphospholipid syndrome (APS). Primary prevention in APS may be aimed at decreasing existing elevated aPL levels, or preventing high aPL titers and/or lupus anticoagulant (LAC) from developing in the first place. Hydroxychloroquine (HCQ) has been shown in retrospective studies to decrease aPL titers in laboratory studies, and to decrease thrombosis risk in patients with systemic lupus erythematosus (SLE). We investigated an association between HCQ use and persistent aPL and/or LAC in SLE.

Methods. We identified all patients over 21 years old with SLE from an urban tertiary care center who had aPL and LAC measured on at least 2 occasions at least 12 weeks apart. We defined the presence of persistent LAC+ and/or at least 1 aPL ≥ 40 U [immunoglobulin A (IgA), IgG, or IgM] as the main outcome variable.

Results. Among 90 patients included in the study, 17 (19%) had persistent LAC+ and/or at least 1 aPL ≥ 40 U. HCQ use was associated with significantly lower odds of having persistent LAC+ and/or aPL ≥ 40 U (OR 0.21, 95% CI 0.05, 0.79, p = 0.02), adjusted for age, ethnicity, and sex.

Conclusion. This is the first study to show that HCQ use is associated with lower odds of having persistently positive LAC and/or aPL. Data from this study provide a basis for the design of future prospective studies investigating the role of HCQ in primary and secondary prevention of APS. (First Release Aug 1 2012; J Rheumatol 2013;40:30–3; doi:10.3899/jrheum.120157)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
ANTIPHOSPHOLIPID ANTIBODIES
LUPUS ANTICOAGULANT
HYDROXYCHLOROQUINE

According to the "2-hit hypothesis," the presence of antiphospholipid antibodies (aPL) is necessary to create a prothrombotic state (first hit). However, aPL alone are not sufficient, and may persist for a long time before the second hit results in the actual thrombotic event[1,2]. Therefore, primary thrombosis prevention may be aimed at decreasing existing elevated aPL, or preventing high aPL titers and/or lupus anticoagulant (LAC) from developing[3].

From the Division of Rheumatology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York, USA.

Supported in part by an Empire Clinical Research Investigator Program Career Development Award to Dr. Broder. Work in Dr. Putterman's laboratory is supported by National Institutes of Health grants RO1 AR048692 and DK090319.

A. Broder, MD, MSc, Assistant Professor, Division of Rheumatology, Albert Einstein College of Medicine, Montefiore Medical Center; C. Putterman, MD, Professor, Chief, Division of Rheumatology, Albert Einstein College of Medicine.

Address correspondence to Dr. A. Broder, Division of Rheumatology, Albert Einstein College of Medicine, F701N, 1300 Morris Park Avenue, Bronx, NY 10461, USA. E-mail: abroder@montefiore.org

Accepted for publication June 6, 2012.

Hydroxychloroquine (HCQ) has been shown to decrease aPL titers in laboratory studies[4,5]. However, only 1 published study to date evaluated the association between HCQ and aPL in a secondary analysis, with a negative result[6]. We investigated whether patients with SLE treated with HCQ were less likely to develop or to maintain persistently positive aPL and/or LAC.

MATERIALS AND METHODS

We included all adult patients with SLE by American College of Rheumatology (ACR) criteria[7] who had LAC, anticardiolipin (aCL), anti-ß$_2$ glycoprotein I (anti-ß$_2$-GPI), and antiphosphatidylserine antibodies measured at least twice, at least 12 weeks apart, between January 2006 and May 2012 at Montefiore Medical Center (MMC), a large urban tertiary care center in Bronx, New York.

Patients were considered to be taking a medication (immunosuppressives, aspirin, HCQ, prednisone, or anticoagulant) if they ever took this medication, similar to previous retrospective studies[6,8]. Race and ethnicity were analyzed as African American/non-African American and Hispanic/non-Hispanic, respectively, based on self-report. Over 90% of non-Hispanics were African American, reflecting the overall racial/ethnic distribution in our center.

Enzyme immunoassay kits (Bio-Rad Laboratories, Hercules, CA,

USA) were used to test aPL. Moderate to high aPL positivity (aPL+) was defined as at least 1 aPL [immunoglobulin G (IgG), IgM, or IgA] ≥ 40 units (moderate/high)[9]. LAC was reported as positive or negative (LAC+/LAC–) by the MMC laboratory in accord with the guidelines of the International Society on Thrombosis and Haemostasis[10].

Because of the retrospective study design, we did not obtain informed consent from the patients, as no identifying information was stored or used in the data analysis. This project was approved by the Institutional Review Board at Albert Einstein College of Medicine/MMC.

Statistical analysis was performed using Stata 12.0 (StataCorp, College Station, TX, USA). No adjustments were made for multiple comparisons in this exploratory study.

RESULTS

The frequencies of aPL and/or LAC among 90 patients included in our study are shown in Table 1. The number of patients who converted from aPL and/or LAC-positive to negative, or from negative to positive, was small.

The results of the bivariate comparisons between patients with persistently positive LAC and/or any aPL ≥ 40 U (n = 17), and patients with either transiently positive or persistently negative LAC and aPL (n = 73) are summarized in Table 2. The 2 groups were similar in age, sex, comorbidity scores, HCQ duration, and disease duration. HCQ use was lower in the persistent aPL/LAC-positive group than in the comparison group, 11 (65%) and 65 (89%), respectively (p = 0.02). The median duration of HCQ use was 49 months (IQR 31, 61) in the aPL/LAC-positive group, and 36 months (IQR 19, 56) in the comparison group (p = 0.6). The minimum duration on HCQ was at least 1 month.

The results of the logistic regression adjusted for age, ethnicity, and sex are shown in Table 3. HCQ use was associated with significantly lower odds of having persistent LAC+ and/or aPL antibodies ≥ 40 U (IgA, IgG, or IgM; OR 0.21, 95% CI 0.05, 0.79, p = 0.02). We did not observe an association between use of other immunosuppressives or prednisone and persistent LAC positivity and/or aPL antibodies ≥ 40 U. Adding these variables to the above model did not change the association between HCQ and LAC/aPL. Age and sex were not independently associated with persistent LAC/aPL.

Similarly, persistently positive LAC and/or moderate/high Sapporo criteria aPL (aCL IgG or IgM, or anti-$ß_2$-GPI IgG or IgM)[9] were associated with HCQ use (OR 0.24, 95% CI 0.06, 0.94, p = 0.04) and Hispanic ethnicity (OR 4.2, 95% CI 1.0, 16.7, p = 0.04; Table 3).

We performed additional analyses to explore a possibility of "by indication" bias with respect to HCQ use in our study, i.e., if HCQ is prescribed for milder SLE, the differences observed in our study may be confounded by SLE severity and multiple comorbidities. When we compared patients taking HCQ (HCQ+) with patients not taking HCQ (HCQ–), no differences were observed with respect to demographics, disease duration, Charlson Comorbidity Score[11], or medications. When we limited our analysis to a subgroup of patients with SLE who were not taking immunosuppressives, presuming less severe SLE (n = 49), HCQ was associated with an OR of 0.17 (95% CI 0.03, 0.88, p = 0.03) of LAC+ and/or Sapporo criteria aPL ≥ 40 U, suggesting that HCQ was independently associated with LAC/aPL positivity.

DISCUSSION

HCQ treatment is currently recommended for patients with SLE who have persistent moderate-high aPL or LAC positivity, for primary prevention (grade 1B to 2B recommendation) based on the other beneficial effects of HCQ in SLE[12,13] and thrombosis[14]. However, ours is the first study to show that HCQ use may be associated with lower odds of having persistently positive LAC and/or aPL in SLE, and therefore may be beneficial in primary prevention.

We also showed that Hispanic ethnicity was associated

Table 1. The frequencies of aPL/LAC positivity in the entire cohort (n = 90). All data are n (%).

Measure	First Measurement	Last Measurement	Both First and Last Measurements
LAC+ and/or at least 1 aPL ≥ 40 U	25 (28)	20 (22)	17 (19)
LAC+ and aPL ≥ 40 U	7 (8)	4 (4)	4 (4)
LAC– and aPL ≥ 40 U	6 (7)	2 (2)	1 (1)
LAC+ and aPL < 40 U	1 (1)	3 (3)	1 (1)
LAC unknown and aPL ≥ 40 U	11 (12)	11 (12)	7 (8)
LAC unknown and aPL < 40 U	37 (41)	28 (31)	24 (27)
At least 1 aPL ≥ 40 U	24 (27)	17 (19)	16 (18)
At least 2 aPL ≥ 40 U	18 (20)	11 (12)	10 (11)
Triple positive (LAC+, and $ß_2$-GPI IgG or IgM ≥ 40 U, and aCL IgG or IgM ≥ 40 U)	7 (8)	3 (3)	3 (3)
Sapporo criteria using moderate/high titers: LAC+, and/or aCL IgG or IgM ≥ 40 U, and/or $ß_2$-GPI IgG or IgM ≥ 40 U	24 (27)	17 (19)	14 (16)

aPL: antiphospholipid antibodies; LAC: lupus anticoagulant; $ß_2$-GPI: $ß_2$-glycoprotein I; IgG: immunoglobulin G; aCL: anticardiolipin; IgM: immunoglobulin M.

Table 2. Baseline characteristics of the study patients.

Characteristics	Persistent LAC and/or aPL ≥ 40 U, n = 17	LAC and aPL Transiently Positive or Negative, n = 73	p*
Age at last aPL/LAC, mean (SD) yrs	41 (16)	39 (13)	0.80
Women, n (%)	14 (83)	69 (95)	0.12
Race, n (%) African American**	3 (27)	41 (64)	**0.02**
Ethnicity, n (%) Hispanic	12 (75)	32 (46)	**0.03**
HCQ, n (%)	11 (65)	65 (89)	**0.02**
Aspirin, n (%)	6 (35)	14 (19)	0.29
Prednisone, n (%)	11 (65)	60 (83)	0.10
Immunosuppressives, n (%)	5 (29)	35 (49)	0.18
Anticoagulation ever for arterial or venous thrombosis or for pregnancy complication, n (%)	7 (41)	13 (18)	**0.02**
Duration on HCQ, mo, median (IQR)	49 (31, 61)	36 (19, 56)	0.38
Time between first and last aPL/LAC measurement, mo, median (IQR)	18 (9, 42)	25 (13, 38)	0.60
Charlson comorbidity, median (IQR)	4.0 (2.0, 7.0)	5.0 (2.0, 7.0)	0.81

* p values in bold type indicate statistical significance. ** Race information not available for 15 patients. aPL: antiphospholipid antibodies; LAC: lupus anticoagulant; HCQ: hydroxychloroquine; IQR: interquartile range.

Table 3. Logistic regression for persistent LAC+ and/or aPL ≥ 40 (IgA, IgG, or IgM) and for persistent positive LAC and/or Sapporo criteria aPL (aCL or ß$_2$-GPI IgG or IgM) ≥ 40 U.

	OR	95% CI	p*
LAC+ and/or aPL ≥ 40 (IgA, IgG, or IgM)			
HCQ use	0.21	0.05, 0.79	**0.02**
Age at last followup	1.0	0.96, 1.0	0.94
Male sex	2.3	0.34, 14.4	0.40
Hispanic ethnicity	3.1	0.84, 11.4	0.09
LAC+ and/or Sapporo criteria aPL (aCL or ß$_2$-GPI IgG or IgM) ≥ 40 U			
HCQ use	0.24	0.06, 0.94	**0.04**
Hispanic ethnicity	4.2	1.0, 16.7	**0.04**

* p values in bold type indicate statistical significance. aPL: antiphospholipid antibodies; LAC: lupus anticoagulant; ß$_2$-GPI: ß$_2$-glycoprotein I; Ig: immunoglobulin; aCL: anticardiolipin; HCQ: hydroxychloroquine.

with higher odds of persistent Sapporo criteria aPL and/or LAC compared with non-Hispanics (predominantly African Americans). While it was previously reported that LAC was more prevalent in whites[15], the association between Hispanic ethnicity and the higher odds of positive aPL and/or LAC has not been previously reported. It is likely that aPL are affected by multiple demographic and SLE-specific factors. Therefore, further studies are needed to investigate the cumulative effect of protective factors and risk factors on developing and maintaining LAC+ and/or elevated aPL.

Our exploratory study has several limitations related to its retrospective design and the sample size, including differential selection, differential and nondifferential misclassification, disease activity, and medication dosage, duration, and compliance. However, we included only patients who satisfied ACR criteria for SLE, and performed several sensitivity analyses, with consistent results. Most importantly, the causal relationship between HCQ use and aPL/LAC positivity could not be established in this cross-sectional study. However, while our study was not designed or powered to evaluate whether HCQ decreased aPL levels, the information we found may be used to design prospective studies to evaluate this important question.

ACKNOWLEDGMENT

We thank Miriam Gordon, PhD, for valuable assistance in editing the manuscript.

REFERENCES

1. Meroni PL, Riboldi P. Pathogenic mechanisms mediating antiphospholipid syndrome. Curr Opin Rheumatol 2001;13:377-82.
2. Bordin G, Boldorini R, Meroni PL. The two hit hypothesis in the antiphospholipid syndrome: Acute ischaemic heart involvement after valvular replacement despite anticoagulation in a patient with secondary APS. Lupus 2003;12:851-3.
3. Pierangeli SS, Erkan D. Antiphospholipid syndrome treatment beyond anticoagulation: Are we there yet? Lupus 2010;19:475-85.
4. Rand JH, Wu XX, Quinn AS, Ashton AW, Chen PP, Hathcock JJ, et al. Hydroxychloroquine protects the annexin A5 anticoagulant shield from disruption by antiphospholipid antibodies: Evidence for a novel effect for an old antimalarial drug. Blood 2010;115:2292-9.
5. Rand JH, Wu XX, Quinn AS, Chen PP, Hathcock JJ, Taatjes DJ. Hydroxychloroquine directly reduces the binding of antiphospholipid antibody-beta 2-glycoprotein I complexes to phospholipid bilayers. Blood 2008;112:1687-95.
6. Erkan D, Derksen WJ, Kaplan V, Sammaritano L, Pierangeli SS, Roubey R, et al. Real world experience with antiphospholipid antibody tests: How stable are results over time? Ann Rheum Dis 2005;64:1321-5.
7. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.

8. Jung H, Bobba R, Su J, Shariati-Sarabi Z, Gladman DD, Urowitz M, et al. The protective effect of antimalarial drugs on thrombovascular events in systemic lupus erythematosus. Arthritis Rheum 2010;62:863-8.
9. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4:295-306.
10. Pengo V, Tripodi A, Reber G, Rand JH, Ortel TL, Galli M, et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost 2009;7:1737-40.
11. Jonsen A, Clarke AE, Joseph L, Belisle P, Bernatsky S, Nived O, et al. Association of the Charlson comorbidity index with mortality in systemic lupus erythematosus. Arthritis Care Res 2011;63:1233-7.
12. Ruiz-Irastorza G, Egurbide MV, Pijoan JI, Garmendia M, Villar I, Martinez-Berriotxoa A, et al. Effect of antimalarials on thrombosis and survival in patients with systemic lupus erythematosus. Lupus 2006;15:577-83.
13. Ruiz-Irastorza G, Ramos-Casals M, Brito-Zeron P, Khamashta MA. Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: A systematic review. Ann Rheum Dis 2010;69:20-8.
14. Petri M. Use of hydroxychloroquine to prevent thrombosis in systemic lupus erythematosus and in antiphospholipid antibody-positive patients. Curr Rheumatol Rep 2011;13:77-80.
15. Petri M. Update on anti-phospholipid antibodies in SLE: The Hopkins Lupus Cohort. Lupus 2010;19:419-23.