

Concise report

Hydroxychloroquine treatment downregulates systemic interferon activation in primary Sjögren's syndrome in the JOQUER randomized trial

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Abstract

Objective. HCQ is frequently used to treat primary SS (pSS), but evidence for its efficacy is limited. HCQ blocks IFN activation, which is present in half of the pSS patients. The effect of HCQ treatment on the expression of IFN-stimulated genes (ISGs) was studied in pSS. Furthermore, HCQ-treated patients were stratified based on IFN activation and differences in disease activity and clinical parameters were studied.

Methods. Expression of ISGs and IFN scores was determined in 77 patients, who were previously enrolled in the placebo-controlled JOQUER trial. Patients were treated for 24 weeks with 400 mg/d HCQ or placebo.

Results. HCQ treatment reduced IFN scores and expression of ISGs compared with the placebo-treated group. HCQ reduced ESR, IgG and IgM levels independently of the patients' IFN activation status. No differences in EULAR SS disease activity index or EULAR SS patient reported index scores were observed after HCQ treatment, even after IFN stratification.

Conclusion. Treatment for 24 weeks with HCQ significantly reduced type I IFN scores and ISG-expression compared with the placebo-treated group. HCQ reduced several laboratory parameters, but failed to improve clinical response. This suggests that in pSS, type I IFN is associated to some laboratory parameters abnormalities, but not related to the clinical response.

Key words: primary Sjögren's syndrome, interferon, hydroxychloroquine

Rheumatology key messages

- Hydroxychloroquine treatment for 24 weeks reduced systemic interferon-stimulated gene expression in SS.
- Hydroxychloroquine treatment for 24 weeks improved ESR, IgG and IgM levels in SS.
- Hydroxychloroquine treatment for 24 weeks did not improve disease activity scores in SS.

Introduction

Primary SS (pSS) is a systemic autoimmune disease with characteristic local dryness of the eyes and mouth. Additionally, systemic complications are frequently observed and include multi-organ involvement and fatigue. There is no cure for pSS and treatment options

focus on symptom relief, prevention of systemic damage and improvement of quality of life. HCQ is an immunomodulatory drug listed as first line treatment in The Sjögren's Syndrome Foundation Clinical Practice Guidelines, and is usually prescribed for arthralgias, myalgias and sometimes for fatigue [1]. Evidence regarding the efficacy of HCQ, however, is limited [2]. The JOQUER randomized placebo-controlled trial (Gottenberg *et al.* [3] JAMA 2014, clinicaltrials.gov identifier NCT00632866) assessed the efficacy of a 24-week HCQ (400 mg/d) treatment in patients with pSS. Disease activity [as assessed by EULAR SS disease activity index (ESSDAI)] and patient-reported symptoms of dryness, pain or fatigue [assessed by the EULAR SS Patient Reported Index (ESSPRI)] did not improve in HCQ-treated pSS patients compared with placebo treatment.

Systemic upregulation of the expression of Toll-like receptor 7, chronic activation of the type I IFN (IFN-I)

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pathway and upregulation of IFN-stimulated genes (ISGs) is present in a subgroup of pSS patients [4, 5]. Among the ISGs are several RNA- and DNA-sensing receptors [6]. Triggering of these sensors by their ligands will result in further stimulation of IFN-I production and induction of a pathogenic loop. HCQ blocks among others TLR7 activation, thereby preventing production of IFNs and induction of ISGs via this route [7]. Here we investigated the effect of HCQ treatment on expression levels of ISGs in whole blood RNA of pSS patients enrolled in the JOQUER trial. Patients were stratified as IFN-I positive or negative. Subsequently, the effect of HCQ treatment on disease activity and objective and subjective clinical parameters in IFN-I positive and negative pSS patients was studied.

Methods

Study design

The study design of the JOQUER trial is described in Gottenberg *et al.* [3]. The protocol was approved by the review board of Hôpital Bichat (Paris, France) and the study was conducted according to the principles of the Declaration of Helsinki. All patients included signed a written informed consent form. In short, between weeks 0 and 24 patients were randomly assigned to receive oral HCQ (400 mg/d) or an indistinguishable placebo. Between weeks 24 and 48 all patients received HCQ, as this drug might be more efficacious after long term usage and is already commonly prescribed in daily practice.

Blood collection and real-time quantitative PCR

Blood was collected at baseline and week 24 in PAXgene tubes for whole blood RNA analysis. Total RNA was isolated from PAXgene tubes (PreAnalytix, Hombrechtikon, Switzerland) and reverse-transcribed to cDNA. For calculation of relative expression, samples were normalized to expression of the household gene *Abl*. Relative expression values were determined from normalized C_T values using the $2^{-\Delta\Delta CT}$ method (User Bulletin, Applied Biosystems).

Calculation of the IFN score

The IFN score was defined by the relative expression of five indicator genes, *IFI44*, *IFI44L*, *IFIT1*, *IFIT3* and *MXA*, as previously described [8]. These five indicator genes were determined in the patient cohort and an external cohort of 55 age- and sex-matched healthy controls (HCs). Mean_{HC} and s.d._{HC} of each indicator gene in the HC group were used to standardize expression levels. The IFN score was calculated per subject representing the sum of these standardized scores. Patients were divided into groups that were positive or negative for systemic IFN-I activation, using a threshold of mean_{HC} + 2 s.d._{HC}.

Questionnaires, laboratory parameters and objective measures of dryness

Acquisition of clinical data and questionnaires is described in Gottenberg *et al.* [3]. In short, the collected questionnaires included: ESSPRI and ESSDAI to study

disease activity; 36-item Medical Outcomes Study Short-Form Health Survey 36 (SF-36) to study quality of life and Hospital Anxiety and Depression Scale to study psychological discomfort. The Schirmer test score and unstimulated salivary flow were measured as objective measures of dryness and several laboratory parameters were included such as serum IgG, IgA and IgM levels, ESR, and CRP measured in each clinical centre.

Statistics

Quantitative variables were described as mean (s.d.) when normally distributed and as median (25th–75th percentile) when non-normally distributed. Constrained longitudinal analysis was used to compare differences in change in values between the two treatment groups for quantitative variables as previously described [3]. Non-parametric analyses of paired data between baseline and the time point of interest was performed using the Wilcoxon signed-rank test. Categorical data were analysed using the chi-square test.

Results

HCQ reduced the IFN score and gene expression of the IFN-I-inducing pathway

A positive IFN signature was observed in 51.9% of the pSS patients at baseline (Supplementary Table S1, available at *Rheumatology* Online). Patients with an IFN signature showed an elevated ESR and higher levels of IgG and IgA (data not shown). Furthermore, patients with an IFN signature had reduced total ESSPRI scores and reduced scores of the pain and fatigue domain of the ESSPRI. At baseline there was no difference in percentage of patients with an IFN signature between the HCQ-treated and placebo group (respectively 51.3% and 52.5%, Supplementary Table S1, available at *Rheumatology* Online). Treatment for 24 weeks with HCQ significantly reduced systemic IFN-I scores in whole blood RNA compared with the placebo group (Table 1) and an increased number of patients were IFN negative after HCQ treatment, while this was not the case in the placebo group (Supplementary Table S1, available at *Rheumatology* Online). In the IFN-I-inducing pathway, systemic levels of TLR9 and MyD88, but not TLR7, were significantly reduced. There was a trend towards lower IRF7 levels, but this did not reach statistical significance. HCQ treatment reduced systemic expression of several ISGs encoding RNA- and DNA-sensing receptors (*IFIH1*, *DDX58*, *EIF2AK2*, *IFI16* and *ZBP1*) compared with the placebo group.

HCQ treatment reduced laboratory parameters, but not disease activity

Differences in HCQ response between patients with or without systemic IFN activation were studied. HCQ reduced ESR, IgG and IgM levels similarly in patient with and without systemic IFN activation (Supplementary Table S2, available at *Rheumatology* online). There was no

TABLE 1 IFN score and molecules in IFN-I pathway, per treated group

Parameter	Placebo		HCQ		Difference in change from baseline score (week 0), adjusted for baseline score, mean (95% CI)	P-value
	n	Mean (s.d.)	n	Mean (s.d.)		
Type I IFN score						
0	40	9.23 (8.22)	37	9.44 (9.20)		
24	40	8.87 (9.66)	36	3.69 (8.64)	-5.24 (-7.76, -2.72)	<0.0001
0, median (25th–75th percentile) ^a	40	11.05 (1.35 to 17.24)	37	12.26 (0.45 to 16.71)		
24, median (25th–75th percentile) ^a	40	12.54 (-0.41 to 16.83)	36	2.63 (-4.75 to 16.71)		
TLR7						
0	40	0.51 (0.21)	37	0.66 (0.34)		
24	40	0.39 (0.20)	36	0.39 (0.16)	-0.05 (-0.13, 0.03)	0.188
TLR9						
0	40	0.57 (0.21)	37	0.59 (0.37)		
24	40	0.38 (0.18)	36	0.29 (0.21)	-0.09 (-0.16, -0.03)	0.004
MyD88						
0	40	24.34 (8.27)	37	21.80 (8.90)		
24	40	22.37 (11.48)	36	16.91 (5.43)	-3.99 (-7.64, -0.35)	0.032
IFIH1						
0	40	1.36 (0.84)	37	1.47 (1.15)		
24	40	1.41 (1.23)	36	0.9 (0.60)	-0.53 (-0.92, -0.13)	0.010
DDX58						
0	40	20.13 (11.25)	37	18.89 (14.59)		
24	40	22.63 (17.18)	36	14.36 (9.90)	-7.34 (-12.43, -2.25)	0.005
IFI16						
0	40	12.70 (5.82)	37	12.36 (6.77)		
24	40	13.87 (9.38)	36	8.76 (4.62)	-4.87 (-7.80, -1.94)	0.001
ZBP1						
0	40	16.33 (9.29)	37	15.95 (11.87)		
24	40	14.95 (10.97)	36	9.07 (6.47)	-5.70 (-8.86, -2.55)	0.001
EIF2AK2						
0	40	7.27 (5.29)	37	7.61 (6.66)		
24	40	11.55 (10.01)	36	6.12 (4.95)	-5.63 (-8.93, -2.34)	0.001
IRF7						
0	38	0.40 (0.41)	36	0.50 (0.73)		
24	40	0.50 (0.74)	36	0.28 (0.28)	-0.23 (-0.50, 0.04)	0.090
0, median (25th–75th percentile) ^a	38	0.29 (0.09–0.54)	36	0.26 (0.10–0.54)		
24, median (25th–75th percentile) ^a	40	0.28 (0.13–0.60)	36	0.18 (0.10 to 0.35)		

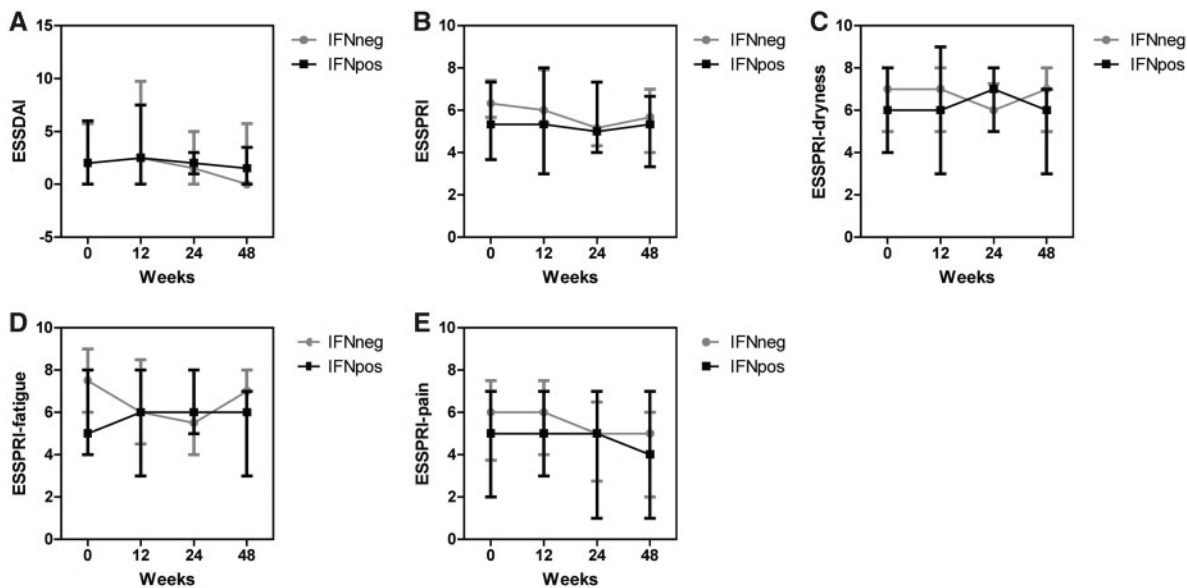
^aWhere the standard deviations exceeded the mean values, medians (25th–75th percentile) are reported as well. IFN-I: type I IFN.

correlation between the change of the IFN score and the change of these secondary outcomes (data not shown). Neutrophil and lymphocyte counts were significantly reduced in the IFN-negative group after HCQ treatment. No difference was observed in the quality of life (assessed by the SF-36) or psychological discomfort (assessed by the Hospital Anxiety and Depression Scale). Additionally, HCQ treatment did not affect ocular or oral dryness measured by Schirmer’s test or unstimulated saliva flow analysis either in IFN-positive or in IFN-negative pSS patients. Neither in the IFN-positive nor in the IFN-negative subgroup did HCQ treatment improve ESSDAI or ESSPRI scores (or its subdomains) (Fig. 1).

Discussion

In this study we showed that HCQ treatment was able to reduce the IFN-I score and ISG-expression in whole blood of patients enrolled in the JOQUER trial. Stratification based upon the presence or absence of IFN activation showed that HCQ reduced ESR, IgG and IgM in patients with and without IFN activation. No effect of HCQ treatment on disease activity was observed, irrespective of the IFN activation status.

HCQ inhibits endosomal Toll-like receptor signalling and previous studies showed that HCQ treatment resulted in a decreased production of IFN α in SLE [9, 10] and

Fig. 1 Disease activity of HCQ-treated pSS patients with ($n = 19$) or without ($n = 18$) IFN activation at baseline

Median values of the EULAR SS Disease Activity Index (ESSDAI) (A), EULAR SS Patients Reported Index (ESSPRI) (B), and its subdomains dryness (C), fatigue (D) and pain (E) are reported at week 0, 12, 24 and 48.

reduced IFN scores in APS [11]. In this study we show that 24-week HCQ treatment reduced IFN-I scores in pSS and downregulated ISG-expression levels. In a cross-sectional study with pSS patients using HCQ for longer periods, we previously observed a trend towards reduced IFN-I scores in the HCQ-users group compared with non-HCQ users, but this did not reach statistical significance [8]. So short term HCQ treatment reduces IFN-I scores, but whether this effect remains after long-term treatment is unclear. An explanation for the variable effect of long-term HCQ treatment could be the possible contribution of pathways other than the Toll-like receptor route that induce IFN expression. Recent data indicate a contribution of RNA- and DNA-sensing pathways to IFN-I production, which are not blocked by HCQ [6]. Therefore, a heterogeneity in induction routes of IFN activation in IFN-positive patients might cause the variable effect of HCQ in this group.

Here the data show that although HCQ decreased the IFN-I signature, there was no effect on disease activity in this pSS cohort. Even in patients with a positive IFN signature, the only effect of HCQ treatment was a reduction of several laboratory parameters. This suggests that some laboratory parameters in pSS might be dependent on IFN-I, but are not related to the clinical response. Interestingly, in a recent positive phase 2 study of baricitinib, an inhibitor of JAK1 and JAK2, in SLE a reduction in the IFN-I signature was observed. But this reduction was not related to the clinical response [12]. This suggests that in SLE the clinical response to baricitinib was not linked to the decrease of the IFN-I signature. Likewise, it was recently announced that the first phase 3 study in SLE with the anti-IFNAR1 antibody anifrolumab failed to achieve its primary end point. Conversely, in the positive

phase 2 study of ustekinumab in SLE there was a positive correlation between the decrease of type II IFN and the clinical response [13].

HCQ is prescribed to patients with mild disease, rather than to patients with severe systemic disease manifestations. This study includes mainly patients with low systemic involvement, reflected in low ESSDAI scores. This is representative for the patient group HCQ is prescribed to in clinical practice. A downside of inclusion of patients with low systemic disease is that it is hard to see improvement of clinical parameters such as measures for disease activity, because they are not highly elevated at baseline. Additionally, it can take up to 6 months before patients notice the effect of HCQ, and hence the treatment time of 24 weeks might be too short.

HCQ is sometimes prescribed to treat fatigue in pSS patients. Pro-inflammatory mechanisms and IFN-I may be linked to fatigue as seen in chronic infections or cancer [14, 15]. However, it is much less clear in autoimmune diseases, and thus in pSS, IFN-positive patients are not more fatigued than IFN-negative patients [8]. In the present study, the decrease of the IFN-I signature after HCQ treatment was not linked to a decrease of fatigue assessed by the ESSPRI. Evidence for the use of HCQ to treat fatigue is weak and mainly based on uncontrolled studies. A recent systematic review on the use of HCQ in pSS reported no effect of HCQ on fatigue [2].

Conclusion

HCQ treatment reduced the IFN-I score and lowered expression levels of several ISGs in whole blood RNA of patients enrolled in the JOQUER trial. Although HCQ

treatment reduced several laboratory parameters, there were no effects on disease activity irrespective of IFN activation status of the patient. This suggests that in pSS IFN-I is associated with some laboratory parameter abnormalities, but not with the clinical response.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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