Hydroxychloroquine reduces IL-6 and pro-thrombotic status

Hydroxychloroquine on pro-thrombotic and pro-inflammatory profile of non-critically ill patients with COVID-19: a *In vivo* and *in vitro* study

Savino Sciascia MD PhD[1], Karen Schreiber MD[2,3,4], Massimo Radin MD[1], Bernhard K. Krämer MD[5], Giuseppe Natale[6], Dario Roccatello MD*[1], Ksenija Stach MD*[5]

(* these authors contributed equally to this work)

1. Center of Research of Immunopathology and Rare Diseases- Coordinating Center of Piemonte and Valle d'Aosta Network for Rare Diseases, Department of Clinical and Biological Sciences, and SCDU Nephrology and Dialysis, S. Giovanni Bosco Hospital and University of Turin.
2. Thrombosis and Haemostasis, Guy's and St Thomas' NHS Foundation Trust, London.
3. Copenhagen Lupus and Vasculitis Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.
4. Danish Institute of Regional Health Research, University of Southern Denmark.
5. Fifth Department of Medicine (Nephrology/Endocrinology/Rheumatology), University Medical Centre Mannheim, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany.
6. Laboratory Department, Giovanni Bosco Hospital, Turin, Italy.

Corresponding Author: Savino Sciascia, MD PhD

Center of Research of Immunopathology and Rare Diseases- Coordinating Center of Piemonte and Valle d'Aosta Network for Rare Diseases, Department of Clinical and

Biological Sciences University of Turin and SCDU Nephrology and Dialysis, S. Giovanni Bosco Hospital, Piazza del Donatore di Sangue 3, 10154, Turin, Italy.

Email savino.sciascia@unito.it Tel +390112402056 Fax +390112402052

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Introduction

The antimalarial drug hydroxychloroquine (HCQ), widely available to treat autoimmune diseases, has been suggested to be a therapeutic tool in the management of COVID-19 patients and reported to be significantly associated with viral load reduction/disappearance (1). However, experimental data in relation to the anti-thrombotic and anti-inflammatory mediated effects of HCQ are still limited.

Methods

For the *in vivo* study, twenty-five PCR-confirmed COVID-19 non-critically ill patients (mean age 61.3± 4.4; 16 male; 22 with upper respiratory tract infection symptoms; 17 with CT-scan confirmed lower respiratory tract infection symptoms; 12 with gastrointestinal manifestations, all with T >37,5°C and Sa02 >93% at admission) were included in a single arm protocol from early March to April 7, 2020, to receive 400 mg of HCQ daily as an add-on therapy to antiviral agents (lopinavir/ritonavir 200/50 mg, bid). Interleukin-6 (IL-6, Elecsys® IL-6 - Diagnostics Roche) levels were assessed before starting HCQ and at day 3 and 7.

In vitro experiments were designed to confirm the clinical findings. Changes in the levels of IL-6 were investigated in the supernatants of human umbilical vein endothelial cells (HUVEC) and human umbilical arterial endothelial cells (HUAEC) induced by pro-inflammatory stimulation with thrombin-activated platelets and tumor necrosis factor-α (TNF-α) as previously described (3,4). In brief, HUVEC and HUAEC were pre-incubated for 24 h with HCQ (concentrations 500, 1000, 2000 ng/ml), corresponding to the range of reported serum levels under HCQ therapy with 400 mg/daily) (2) and then stimulated for 1 hour with TNF-α in direct contact with thrombin activated platelets to mimic a pro-inflammatory and pro-thrombotic condition. After incubation the expression of urokinase plasminogen activator receptor (uPAR) and Intercellular Adhesion Molecule 1 (ICAM-1) on endothelial

cells were measured by flow cytometry. This study was conducted in accordance with the Helsinki Declaration and the protocol was approved by the local committee for off-label drug use.

Results

In the *in vivo* experiments, we observed a significant reduction in the IL-6 levels after the beginning of treatment with HCQ (IL-6 baseline media [SEM] 597.7 ± 495.3 pg/ml; day 3: 369.7 ± 275.1 pg/ml; day 7: 114.3 ± 57.5 pg/ml, $p < 0.05$) (Figure 1). Ferritin levels also showed a trend in reduction, albeit not statistically significant (baseline media [SEM] 1181.5 ± 244.4 ng/ml; time 1: 1190.1±187.7 ng/ml; time 3: 907.0 ± 216.6 ng/ml, p=0.1898). *In vivo* results were paralleled by the *in vitro* experiments. The *in vitro* experiments were performed on HCQ pre-incubated HUAECs under inflammatory conditions and showed a dose dependent IL-6 reduction (as already observed in HUVEC, data not shown) (Figure 1.B) as well as a reduction of expression of uPAR and ICAM-1 (Figure 2).

Discussion

Very recently Gautret et al. (1) observed that HCQ was associated with an early (three to six days) clearing viral nasopharyngeal carriage of COVID-19 patients. A significant difference was found between HCQ-treated patients and controls starting even on day 3 post-inclusion. These clinical results were in line with the *in vitro* findings by Yao et al. (5), demonstrating that both chloroquine and HCQ inhibit COVID19 in vitro, with HCQ found to be more potent than chloroquine. When analysing predictors of mortality in a recent retrospective multicentre study of 150 confirmed COVID-19 cases in Wuhan, China, Ruan and colleagues found elevated IL-6 levels, among inflammatory serological markers, were predictors of a fatal outcome, suggesting that mortality might be due to virally driven “cytokine storm syndrome” (6). Our observation suggests that the use of HCQ in non-

critically ill patients might represent a pragmatic approach to reduce the hyperinflammation and the pro-thrombotic status, with consequent positive impact on prognosis. The lack of control groups, small sample size and the use of concomitant antiviral therapy may limit the extrapolability of our results. Well-designed clinical studies are urgently needed.

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LEGEND

Figure 1. Colum bar graph showing a significant reduction in the IL-6 levels after the beginning of treatment with HCQ (baseline; day 3; day 7).

Figure 2. PANEL A. Concentrations of IL-6 in the supernatants of thrombin-prestimulated platelets enhanced human umbilical arterial endothelial cells (HUAEC) assed by enzyme linked immunosorbent assay for soluble IL-6 with and without 24 h pre-incubation with 500/1000/2000 ng/ml HCQ. PANEL B. Expression of ICAM-1 (PANEL B) and uPAR (PANEL C) on HUAEC with and without 24 h pre-incubation with 500/1000/2000 ng/ml HCQ expressed by mean fluorescence intensity arbitrary unit

PLT = platelets; TH= thrombin; TNFalpha = tumor necrosis factor alpha

(* $p < 0.05$, as evaluated by Dunnett's test)