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CLINICAL PHARMACOLOGY

Pharmacokinetics and pharmacodynamics of hydroxychloroquine in hospitalized patients with COVID-19

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Received 17 November 2020; accepted 22 January 2021
Available online 28 January 2021

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https://doi.org/10.1016/j.therap.2021.01.056
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Summary

Background.— Hydroxychloroquine (HCQ) dosage required to reach circulating levels that inhibit SARS-CoV-2 are extrapolated from pharmacokinetic data in non-COVID-19 patients.

Methods.— We performed a population-pharmacokinetic analysis from 104 consecutive COVID-19 hospitalized patients (31 in intensive care units, 73 in medical wards, n = 149 samples). Plasma HCQ concentration were measured using high performance liquid chromatography with fluorometric detection. Modelling used Monolix-2019R2.

Results.— HCQ doses ranged from 200 to 800 mg/day administered for 1 to 11 days and median HCQ plasma concentration was 151 ng/mL. Among the tested covariates, only bodyweight influenced elimination oral clearance (CL) and apparent volume of distribution (Vd). CL/F (F for unknown bioavailability) and Vd/F (relative standard-error, %) estimates were 45.9 L/h (21.2) and 6690 L (16.1). The derived elimination half-life (t1/2) was 102 h. These parameters in COVID-19 differed from those reported in patients with lupus, where CL/F, Vd/F and t1/2 are reported to be 68 L/h, 2440 L and 19.5 h, respectively. Within 72 h of HCQ initiation, only 16/104 (15.4%) COVID-19 patients had HCQ plasma levels above the in vitro half maximal effective concentration of HCQ against SARS-CoV-2 (240 ng/mL). HCQ did not influence inflammation status (assessed by C-reactive protein) or SARS-CoV-2 viral clearance (assessed by real-time reverse transcription-PCR nasopharyngeal swabs).

Conclusion.— The interindividual variability of HCQ pharmacokinetic parameters in severe COVID-19 patients was important and differed from that previously reported in non-COVID-19 patients. Loading doses of 1600 mg HCQ followed by 600 mg daily doses are needed to reach concentrations relevant to SARS-CoV-2 inhibition within 72 hours in ≥ 60% (95% confidence interval: 49.5—69.0%) of COVID-19 patients.

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Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| ALAT         | alanine aminotransferase |
| ASAT         | aspartate aminotransferase |
| β            | covariate effect parameter |
| BMI          | body mass index |
| BW           | bodyweight |
| CL/F         | apparent elimination clearance |
| CNIL         | National Commission on Informatics and Liberties |
| COVID-19     | novel coronavirus disease 2019 |
| CRP          | C-reactive protein |
| CYP          | cytochrome P450 |
| EC50         | half maximal effective concentration |
| F            | bioavailability |
| η            | between-subject variability |
| HCQ          | hydroxychloroquine |
| Ht           | hematocrit |
| Ka           | absorption rate constant |
| MDRD         | modification of diet in renal disease equation |
| QTc          | corrected QT interval |
| RE           | rheumatoid arthritis |
| RT-PCR       | reverse transcription polymerase chain reaction |
| SARS-CoV-2   | severe acute respiratory syndrome coronavirus |
| SLE          | systemic lupus erythematosus |
| T.i.d        | ter in die |
| U-HPLC       | ultra-high performance liquid chromatography |
| V/F          | apparent volume of distribution |
| σr           | proportional residual variability |

Introduction

A new human respiratory-tropic coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV-2), has spread rapidly worldwide. Novel coronavirus disease 2019 (COVID-19), the disease caused by this virus, has a very variable clinical presentation, ranging from pauci-symptomatic to acute respiratory distress syndrome. Several drugs are being evaluated for the treatment of covid-19 including hydroxychloroquine (C18H26ClN3O) [HCQ]. Some observational, non-randomized studies have suggested the possible efficacy of HCQ associated or not with azithromycin in COVID-19 patients contrasting with other studies [1—5]. Recent randomized controlled trials showed that HCQ was not effective in hospitalized or non-hospitalized patients with COVID-19 [6—9]. The results of over 120 randomized controlled trials for the treatment and prevention of COVID-19 are pending. Doses of HCQ tested were highly variable, ranging from 400 mg/day for few weeks up to 2.4 g on day 1 as a loading dose followed by 400 mg/day for few days, based on extrapolation from pharmacokinetics properties of HCQ derived from its approved indications (malaria, auto-immune diseases) [10]. Yao et al. reported that HCQ possesses antiviral activity, against SARS-CoV2 in vitro [11] with an EC50 (half maximal effective concentration) of 0.72 μM (240 ng/mL) of HCQ on Vero-cells. The antiviral effect of HCQ, has been suggested to result from increasing intracellular pH leading to decreased phago-lysosome fusion, and impaired viral receptor glycosylation. Moreover, HCQ has immune-modulating
effect by inhibiting toll-like receptor signaling, decreasing production of cytokines, especially IL-1 and IL-6, potentially mitigating the cytokine release syndrome induced by SARS-CoV-2 infection [12–14].

Steady-state pharmacokinetics of HCQ has previously been reported in healthy volunteers, adult patients with malaria [15], systemic lupus erythematosus (SLE) [16,17] and rheumatoid arthritis (RA) [18–20] and are summarized in Table 1. Herein, we analyzed plasma and blood concentration data in a cohort of consecutive patients hospitalised with COVID-19 who received HCQ. The aim of this work was to characterize HCQ pharmacokinetics in the setting of COVID-19 and to identify its main influencing covariates. The pharmacokinetic model developed from COVID-19 patients then allowed us to determine the best HCQ dosing regimen to rapidly reach relevant theoretical antiviral concentrations, i.e. higher than HCQ EC50 on SARS-CoV-2. We finally analyzed if there was any HCQ dose-efficacy relationship on SARS-CoV-2 clearance and inflammation parameters.

### Materials and methods

We conducted a monocenter study in consecutive patients with confirmed COVID-19 (positive for SARS-CoV-2) with reverse transcription polymerase chain reaction (RT-PCR), sampled for HCQ therapeutic drug monitoring left at the discretion of the treating physicians. Patients were treated with oral hydroxychloroquine sulfate (Plaquenil®, Sanofi-Winthrop, Paris, France). Concentrations of HCQ and its metabolites in whole blood and plasma were assayed by ultra-high performance liquid chromatography (U-HPLC) with fluorometric detection [21]. This retrospective study was based on data extracted from medical records, in strict compliance with the French reference methodology MR-004, established by French National Commission on Informatics and Liberties (CNIL) and was approved by Sorbonne University ethics Committee (CER-2020-14-JOCOVID).

### Pharmacokinetic-dynamic modelling

Hydroxychloroquine time courses were analyzed using the nonlinear mixed effect modelling software program Monolix 2019R2 [22]. To ensure full convergence of the program, the iteration number was fixed to 1000 with 50 Markov Chain Monte Carlo. The effect of the demographic and clinical characteristics which were thought to influence pharmacokinetics were evaluated for the following covariates: bodyweight (BW), height, age, sex, hepatic function using alanine aminotransferase (ALAT), creatinine clearance using modification of diet in renal disease equation (MDRD), C-reactive protein (CRP) level, serum albumin, co-prescription with azithromycin or other macrolides, intensive care unit vs. medical wards patients, platelets/white cells counts and hematocrit. Parameter estimates were standardized for a mean standard covariate using an allometric model:

\[
P_i = P_{STD} \times (\text{COVi/COViSTD})^{PWR}
\]

where PSTD is the standard value of parameter and Pi and COVi are the parameter and covariate values of the ith individual. The superscript PWR denotes an exponent power.

For bodyweight, allometric scaling theory dictates that PWR are typically 1 and 0.75 for volumes and clearance terms, respectively [23]. The goodness-of-fit of each model was evaluated by the observed-predicted (population and individual) concentration scatter plots, by the visual inspection of the individual concentration-time courses, and the prediction-corrected visual predictive checks.

A one-compartment open model best described HCQ pharmacokinetics, whatever the sampling reference, blood or plasma. The parameters of the model were the elimination oral clearance (CL/F), the apparent volume of distribution (V/F) and the absorption rate constant, Ka (with F, as the unknown bioavailability). Given the lack of data on the absorption phase, Ka was fixed to 0.75 and 1.15 h⁻¹ in blood and plasma respectively as previously.
Table 2  Demographic and biological characteristics of 104 patients.

| Characteristic                     | Mean ± standard deviation | Minimum | Maximum |
|------------------------------------|---------------------------|---------|---------|
| Age, years                         | 63.0 ± 14.4               | 25      | 99      |
| Weight, kg                         | 79 ± 16                   | 40      | 150     |
| Height, cm                         | 169 ± 11                  | 146     | 192     |
| BMI, kg/m²                         | 27.3 ± 5.0                | 17.8    | 51.9    |
| Sex, (female) %                    | 32                        | NA      | NA      |
| Patient in intensive care, %       | 23                        | NA      | NA      |
| MDRD, mL/min/1.73 m²               | 86.0 ± 33.6               | 5       | 194     |
| Creatinine, µmol/L                 | 98 ± 82                   | 34      | 808     |
| Albumin, g/L                       | 29.0 ± 6.9                | 12      | 63      |
| HT, %                              | 35.0 ± 6.5                | 18      | 49      |
| Platelet, 10⁹ L                    | 313 ± 134                 | 52      | 753     |
| White blood cells, 10⁹ L           | 7.5 ± 4.7                 | 2       | 32.4    |
| C-reactive protein (CRP), mg/L     | 86 ± 98                   | 2       | 469     |
| ASAT, U/L                          | 69 ± 74                   | 11      | 486     |
| ALAT, U/L                          | 63 ± 49                   | 13      | 252     |
| Dose HCQ, mg/day                   | 563 ± 99                  | 200     | 800     |
| Observation duration, days         | 5.3 ± 2.3                 | 1       | 12      |
| Blood concentration                |                          |         |         |
| HCQ, ng/mL                         | 586 ± 457                 | 50      | 2792    |
| Plasma concentration               |                          |         |         |
| HCQ, ng/mL                         | 193 ± 152                 | 12      | 795     |
| HCQBlood/HCQPlasma                 | 4.0 ± 2.3                 | 1       | 15      |

ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; BMI: body mass index; HCQ: hydroxychloroquine; HT: hematocrit; NA: non-applicable; MDRD: modification of diet in renal disease equation.

All data were collected at the time of HCQ sampling.

reported [23]. Between-subject variabilities were estimated for CL/F and V/F parameters and the residual variability was described by a proportional model. F stands for unknown bioavailability.

HCQ and viral clearance

Different covariates, including HCQ concentration, thought to influence the time-to-PCR negativation were tested using the R-program [24] and the survival package [25]. The Kaplan–Meier method and log-Rank test were used for this purpose. Patients were split according to their individual model-predicted HCQ plasma concentration at 48 h using the 1st, 50th or 75th quartile. Thereafter, two Kaplan–Meier curves were generated for each splitting factor. The time to negativation was the first occurrence when two successive RT-PCR were negative.

HCQ effect on CRP

The CRP time courses were modelled as a function of time and plasma HCQ concentration (Cp) as:

\[
\text{CRP} = \text{CRP}_0 \cdot \left(1 - f_{\text{HCQ}} \cdot \frac{\text{Cp}}{(\text{Cp}_{50} + \text{Cp})} - (1 - f_{\text{HCQ}})\right) \cdot t / (t + t_{50})
\]

where CRP₀, f_HCQ, Cₚ₅₀ and tₕ₀ denote the initial CRP concentration, fractional effect of HCQ, HCQ concentration or time that produce a 50% decrease in the CRP₀ level. The model stands for the effect of HCQ (f_HCQ and Cₚ₅₀) plus an independent time-related effect ([1 – f_HCQ] and tₕ₀), which simultaneously decrease the initial CRP₀ level.

Results

Demographic and biological characteristics

A total of 149 plasma samples were obtained from 104 COVID-19 patients, (n = 31 in intensive care units and n = 73 in medical wards). Time point of drug sampling was performed at various times after HCQ dosing, i.e., mean 16.2 h (SD 30 h). Characteristics of included COVID-19 patients are detailed in Table 2. At the time of HCQ blood sampling, 10/104 patients (9.6%) had severe renal failure with a glomerular filtration rate < 30 mL/min/1.73 m² and 34/104 (32.7%) had ALAT levels 3 times higher than the upper normal limit. In all patients, SARS-CoV-2 was confirmed by a positive (RT-PCR) assay on a nasopharyngeal sample. All patients were treated with HCQ and 75/104 (72%) had a post-treatment follow-up with RT-PCR on nasopharyngeal samples. HCQ was combined with a macrolide antibiotic in 29 patients (n = 6 with azithromycin). The mean time between the introduction of HCQ and the onset of symptoms was 8.6 ± 5 days. The usual HCQ dosage was 200 mg t.i.d (78/104 patients) for 1 to 11 days (3 patients received an 800 mg loading dose). Fig. 1 shows plasma and blood HCQ concentrations available in our cohort.
Pharmacokinetic modelling

The population plasma and blood HCQ pharmacokinetic parameter estimates and their influencing covariates are summarized in Tables 3 and 4, respectively. These parameter estimates were different from those reported in other diseases (lupus, malaria, rheumatoid arthritis) or in healthy volunteers (Table 1). Fig. 2A shows the visual predictive checks for the HCQ plasma final model in COVID-19 (for blood final model, see Fig. 2B). The observed concentrations percentiles are well included in the corresponding model-predicted 90% confidence interval bands. Among the tested covariates (age, bodyweight, gender, hepatic and renal function, CRP, intensive care vs. medical wards care, macrolide/azithromycin co-prescription, platelet count), bodyweight (based on allometry principles) was the sole variable having an effect on plasma or blood HCQ CL/F and V/F prediction that improved the model. Platelet count had an additional significant effect on V/F estimation for blood HCQ (Table 4).

Relying on our final pharmacokinetics parameters modelling, we generated representative plasma HCQ concentrations-time courses using various dosing regimens of major COVID-19 prospective trials testing HCQ (Fig. 3A). Concentration vs. time profiles were also drawn according to documented plasma HCQ pharmacokinetics parameters estimates (Table 1) derived from healthy volunteers, lupus and malaria patients (Fig. 3B–D, respectively). Depending on the diseases-specific estimates used, results were dramatically different. Fig. 4 shows 4 dosing regimens based on our COVID-19 plasma HCQ pharmacokinetics estimates leading to HCQ plasma concentration above the HCQ EC50 against SARS-CoV-2 value 48 to 72 h after treatment initiation. Day 1 loading doses of HCQ ≥ 1600 mg followed by daily dose ≥ 600 mg reached theoretical concentrations in ≥ 40% (95% confidence interval 30–50%) and ≥ 60% (95% confidence interval: 49.5–69.0%) of COVID-19 patients within 48 and 72 hours, respectively, assuming a distribution of body weights generally similar to that of our population. For a selected dosing scheme, effect of 1st and 3rd body weight quartiles on CL and Vd population parameters are shown in Fig. 5A and HCQ plasma concentration-times courses for patients weighing 79 kg (median bodyweight) using their individualized pharmacokinetic parameters are depicted in Fig. 5B. An important between patient’s variability, leading to low or unexpectedly high (potentially toxic) HCQ plasma concentrations, ensues despite administering a standardized HCQ dosing (Fig. 5B).
Figure 2. Prediction-corrected visual predictive check for plasma (A) and blood (B) hydroxychloroquine population pharmacokinetics. Plain (●) and green lines stand for prediction-corrected observed concentrations and their 5th, 50th and 95th percentiles. Light blue and red bands stand for the corresponding model-predicted 90% confidence intervals.

Figure 3. Representative predicted plasma hydroxychloroquine (HCQ) concentrations-time courses as a function of the dosing regimen evaluated in major prospective trials testing HCQ for COVID-19. Curves are drawn according to our final parameters for a typical patient weight (WT) of 79 kg (observed median). Dosing schedules are 2.4 g loading dose then 400 mg/12 h (RECOVERY), 1.2 g loading dose then 200 mg/8 h (SANOFI), 200 mg/8 h (IHU Marseille) and 800 mg loading dose then 400 mg/24 h (DISCOVERY). Curves shown are using COVID-19 patients (A), lupus patients (B), malaria patients (C), and healthy subjects (D) parameters.
**Table 3** Median plasma hydroxychloroquine population pharmacokinetic parameters in 104 COVID-19 adult patients.

| Parameter       | Estimate | %res |
|-----------------|----------|------|
| ka, h^-1        | 1.15     | Fixed|
| V/F, L          | 6690     | 16.1 |
| β, V/F*(BW/70)  | 1        | Fixed|
| CL/F            | 45.9     | 21.2 |
| β, CL/F*(BW/70) | 0.75     | Fixed|
| γV/F            | 0.61     | 18.9 |
| γCL/F           | 0.69     | 25.1 |
| σ, ng/mL        | 64.1     | 9.76 |

β: covariate effect parameter; η: between-subject variability; α: proportional residual variability; BW: body weight; COVID-19: novel coronavirus disease 2019; CL/F: apparent elimination clearance; F: unknown bioavailability; Ka: absorption rate constant; V/F: apparent volume of distribution. BW: CL/F and V/F estimates are normalized to a 70 kg BW, i.e., for the ith patient CL/Fi = CL/F*(BWi/70)0.75 and V/Fi = V/F*(BWi/70)*(PLATi/300,000)−0.726.

**Table 4** Median blood hydroxychloroquine population pharmacokinetic parameters in 98 COVID-19 adult patients.

| Parameter       | Estimate | %res |
|-----------------|----------|------|
| ka, h^-1        | 0.75     | fixed|
| V/F, L          | 1.990    | 15.9 |
| β, V/F*(BW/70)  | 1        | fixed|
| β, V/F*(PLAT/300,000)  | −0.726  | 37   |
| CL/F            | 14.7     | 13.5 |
| β, CL/F*(BW/70) | 0.75     | fixed|
| γV/F            |          |      |
| γCL/F           |          |      |
| σ, proportional | 0.272    | 12.2 |

β: covariate effect parameter; η: between-subject variability; α: proportional residual variability; BW: body weight; CL/F: apparent elimination clearance; COVID-19: novel coronavirus disease 2019; F: unknown bioavailability; Ka: absorption rate constant; V/F: apparent volume of distribution. BW: CL/F and V/F estimates are normalized to a 70 kg BW plus V/F to a 300,000 platelets count, i.e., for the ith patient CL/Fi = CL/F*(BWi/70)0.75 and V/Fi = V/F*(BWi/70)*(PLATi/300,000)−0.726.

**Discussion**

In this study, we developed a plasma and blood population pharmacokinetics models of HCQ based on data obtained in hospitalized COVID-19 patients in intensive care units and in medical wards. The blood and plasma pharmacokinetics were described by a one-compartment model with first-order absorption. Body weight had a significant effect on CL and Vd in both matrices. HCQ pharmacokinetic parameters in COVID-19 patients are different from those of other pathologies (lupus, malaria, rheumatoid arthritis) and healthy volunteers [15,16,20]. The theoretical ideal lowest dose to achieve a target plasma concentration >EC50 (240 ng/mL) within 48/72 hours in most patients was 1600 mg as a loading dose, followed by 200 mg/8 h thereafter. Nevertheless, plasma concentrations of HCQ showed a high interindividual variability (Fig. 1) mainly influenced by body weight. In COVID 19, either HCQ dosage adjusted on body weight or HCQ plasma therapeutic drug monitoring may be useful options if HCQ is clinically effective on COVID-19. However, in our cohort study, there was no significant influ-
ence of HCQ plasma concentrations on inflammation (CRP) or on viral clearance (RT-PCR).

Interestingly, recent studies used HCQ pharmacokinetics parameters derived from autoimmune diseases, to propose dosing regimen of HCQ to be used in COVID-19 patients [26–28]. Thus, our data suggest that relevance of these types of modelling might be toned down given the importance of difference observed between HCQ pharmacokinetic parameters in COVID-19 versus other settings (Table 1). Supporting our findings, preliminary pharmacokinetics data from a small cohort of 7 hospitalized COVID-19 patients treated with HCQ as part of the RECOVERY trial (2.4 g as loading dose then 400 mg/12 h) have recently been published [29]. The results indicate that HCQ concentrations are lower than those expected based on previous modelling, even though a high dose regimen was used.

Of note, our PK blood parameters estimates were concordant with those estimated by Thémans et al. [30] and other groups [28,30,31] providing evidence that a high HCQ loading dose is needed to reach circulating levels in COVID-19 patients theoretically relevant as compared to in vitro SARS-CoV-2 inhibitory concentrations.

In our cohort including over 100 COVID-19 patients, subjects had different profiles ranging from hospitalization in medicine to intensive care unit, with variable renal and hepatic functions, as well as co-prescription with macrolides, most of which are cytochrome P-450 inhibitors [32]. None influenced HCQ plasma and blood pharmacokinetics in COVID-19 except weight, or weight and platelet count, respectively. This finding is concordant with other HCQ pharmacokinetic studies in lupus and malaria settings, in which body mass index and platelet count were also significant contributing covariates in the model [16,33].

Of note, the relationship between circulating concentrations of HCQ and clinical efficacy has been demonstrated in rheumatoid arthritis and systemic lupus erythematosus [17–19,34]. However, our study did not show any association between plasma HCQ concentration and time to negativation of SARS-CoV-2 viral load in hospitalized patients, or resolution of inflammation (assessed by CRP). We are currently studying the association between the blood and plasma concentration of HCQ and QTc (i.e. the duration of ventricular repolarization corrected for heart rate, a predictor of ventricular arrhythmias) [27] in patients with COVID-19 to further assess cardiovascular safety of HCQ in COVID-19 setting [35]. Indeed, the risks of cardiotoxicity associated with HCQ during the COVID-19 pandemic might increase for several reasons. Patients with COVID-19 have multiple risk factors for drug-induced QT prolongation and proarrhythmia: hypokalemia; fever amplifying drug-induced IKr blockade; and an increase in interleukin-6, as seen in COVID-19 infection which has been suggested as a mechanism of the QT prolongation associated with inflammation [36]. The French Pharmacovigilance Network has reported 103 notifications of cardiac adverse drug reactions associated with “off-label” use of hydroxychloroquine since March 2020 up to April 2020 [37]. These observations, on top of its lack of efficacy, justified limiting the prescription of HCQ in COVID-19 patients [38].

The retrospective, observational design of our work is the main limitation. The blood and nasopharyngeal samples were not systematically assessed for all patients during the treatment period. This may have biased our results by precluding to demonstrate that there was an association between plasma HCQ levels and negative viral loads. Fortunately, the detailed time course of viral load was unknotted, precluding further analysis. However, multiple lines of evidence are emerging against HCQ efficacy in hospitalized COVID-19, even with theoretically effective high dosing regimen such as in the RECOVERY randomized controlled trial [39–41]. In that study, patients received a loading dose of 2.4 g then 400 mg every 12 hours. HCQ was not associated with reduced mortality but was associated with an increased length of hospital stay and a trend towards increased risk of progression to invasive mechanical ventilation or death [36,42]. Indeed, the dosing regimen used in the RECOVERY trial was even higher than the adapted dosing regimen that we can recommend based on in vitro HCQ EC50.

Figure 5. Mean plasma hydroxychloroquine (HCQ) concentration-time courses for a patient with bodyweight (WT) <58 kg or >103 kg and half-life >70 h, red and blue curves, respectively (A) and for typical patients with 79 kg WT and clearance (CL) ranging between 30–68 L/h and volume of distribution (Vd) between 4765–13,470 L, black curves drawn from variable CL and Vd Bayesian estimates (B). Dosing regimen is 200 mg HCQ/8 h, with no loading dose.
Figure 6. Time-to-Sars-Cov-2 PCR negativation curves as a function of hydroxychloroquine (HCQ) plasma levels within 48 hours of HCQ start. Blue and red curves represent patients with an HCQ plasma concentration at 48 h below or above the 1st HCQ plasma concentration quartile observed in our cohort, respectively (72 ng/mL, A), median (95 ng/mL, B) and 3rd quartile (129 ng/mL, C).
on SARS-CoV-2 and HCQ human pharmacokinetic parameters in COVID-19, identified in this work.

Conclusions
Interindividual variability of HCQ pharmacokinetics parameters in hospitalized COVID-19 patients was important and parameters differed from those identified in non-COVID-19 patients. No effect of HCQ was found on SARS-CoV-2 (nasopharyngeal) viral clearance nor on inflammation resolution. Loading doses of 1600 mg HCQ followed by 600 mg daily doses reached within 72 hours, concentrations relevant to SARS-CoV-2 inhibition in ≥60% (95% confidence interval: 49.5—69.0%) of COVID-19 patients.

Funding
This research received no external funding.

Disclosure of interest
The authors declare that they have no competing interest.

References
[1] Arshad S, Kilgore P, Chaudhry ZS, Jacobsen G, Wang DD, Huizing K, et al. Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. Int J Infect Dis 2020;97:396—403.
[2] Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020;56(1):105949.
[3] Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow-up: a pilot observational study. Travel Med Infect Dis 2020;34:101663.
[4] Mikami T, Miyashita H, Yamada T, Harrington M, Steinberg D, Dunn A, et al. Risk factors for mortality in patients with COVID-19 in New York City. J Gen Intern Med 2020;1—10. http://dx.doi.org/10.1007/s11606-020-05983-z.
[5] Million M, Lagier JC, Gautret P, Colson P, Fournier PE, Amran S, et al. Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: a retrospective analysis of 1061 cases in Marseille, France. Travel Med Infect Dis 2020;35:101738.
[6] Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hirpiscak G, et al. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. N Engl J Med 2020;382(25):2411—8.
[7] Rosenberg ES, Dufort EM, Udo T, Wilberschied LA, Kumar J, Tesoriero J, et al. Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York State. JAMA 2020;323(24):2493—502.
[8] Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, et al. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. N Engl J Med 2020;383(6):517—25.
[9] Tang W, Cao Z, Han M, Wang Z, Chen J, Sun W, et al. Hydroxychloroquine in patients with mainly mild to moderate coronavirus disease 2019: open label, randomised controlled trial. BMJ 2020;369:m1849.
[10] Le MP, Peiffer-Smadel N, Guedj J, Neant N, Mentre F, Ader F, et al. Rationale of a loading dose initiation for hydroxychloroquine treatment in COVID-19 infection in the DisCoVery trial. J Antimicrob Chemother 2020;75(9):2376—80.
[11] Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis 2020;71(15):732—9.
[12] Akpovwa H. Chloroquine could be used for the treatment of filoviral infections and other viral infections that emerge or emerged from viruses requiring an acidic pH for infectivity. Cell Biochem Funct 2016;34(4):191—6.
[13] Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today’s diseases? Lancet Infect Dis 2003;3(11):722—7.
[14] Rossi B, Nguyen LS, Zimmermann P, Boucenna F, Dubret L, Baucher L, et al. Effect of tocilizumab in hospitalized patients with severe COVID-19 pneumonia: a case-control cohort study. Pharmaceuticals (Basel) 2020;13(10):317.
[15] Lim HS, Im JS, Cho JY, Baes K, Klein TA, Yeom JS, et al. Pharmacokinetics of hydroxychloroquine and its clinical implications in chemophrophylaxis against malaria caused by Plasmodium vivax. Antimicrob Agents Chemother 2009;53(4):1468—75.
[16] Morita S, Takahashi T, Yoshida Y, Yokota N. Population pharmacokinetics of hydroxychloroquine in Japanese patients with cutaneous or systemic lupus erythematosus. Ther Drug Monit 2016;38(2):259—67.
[17] Costedoat-Chalumeau N, Amoura Z, Hulot JS, Hammoud HA, Aymard G, Cabouve 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.
[18] Tett SE, Cutler DJ, Beck C, Day RO. Concentration–effect relationship of hydroxychloroquine in patients with rheumatoid arthritis—a prospective, dose ranging study. J Rheumatol 2000;27(7):1656—60.
[19] Munster T, Gibbs JP, Shen D, Baethge BA, Botstein GR, Caldwell J, et al. Hydroxychloroquine concentration–response relationships in patients with rheumatoid arthritis. Arthritis Rheum 2002;46(6):1460—9.
[20] Carmichael SJ, Charles B, Tett SE. Population pharmacokinetics of hydroxychloroquine in patients with rheumatoid arthritis. Ther Drug Monit 2003;25(6):671—81.
[21] Noe G, Amoura Z, Com barel D, Lori L, Tissot N, Seycha A, et al. Development and validation of a fast ultra-high performance liquid chromatography–fluorescent method for the quantification of hydroxychloroquine and its metabolites in patients with lupus. Ther Drug Monit 2019;41(4):476—82. http://dx.doi.org/10.1097/FTD.0000000000000614.
[22] http://www.lxofit.eu [Accessed January 25, 2021].
[23] Anderson BJ, Holford NH. Mechanism-based concepts of size and maturity in pharmacokinetics. Annu Rev Pharmacol Toxicol 2008;48:303—32.
[24] R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2018. https://www.R-project.org/ [Accessed January 25, 2021].
[25] Therneau T. A package for survival analysis in S. version 2.38; 2015 https://CRAN.R-project.org/package=survival [Accessed January 25, 2021].
[26] Martin-Blondel G, Ruiz S, Murriss M, Fager S, Duhadle V, Eyvrad F, et al. Hydroxychloroquine in COVID-19 patients: what still needs to be known about the kinetics. Clin Infect Dis 2020;71(11):2962—4.
[27] Funck-Brentano C, Salem JE, Nguyen LS, Drici MD, Roden DM. Response to the editorial “COVID-19 in patients with cardiovascular diseases”: Covid-19 treatment with hydroxychloroquine or chloroquine and azithromycin: a potential risk of Torsades de Pointes. Arch Cardiovasc Dis 2020;113(5):367–8.

[28] Garcia-Cremades M, Solans BP, Hughes E, Ernest JP, Wallender A, Awekeka F, et al. Optimizing hydroxychloroquine dosing for patients with COVID-19: an integrative modeling approach for effective drug repurposing. Clin Pharmacol Ther 2020;108(2):253–63.

[29] MacGowan AP, Hamilton F, Bayliss M, Read L, Attwood M, Noel A, et al. Hydroxychloroquine serum concentrations in non-critical care patients infected with SARS CoV 2. medRxiv 2020, 2020.06.23.20137992. https://www.medrxiv.org/content/10.1101/2020.06.23.20137992v1. [Accessed January 25, 2021].

[30] Themans P, Belkhir L, Dauby N, Yombi JC, De Greef J, Delongie KA, et al. Population pharmacokinetics of hydroxychloroquine in COVID-19 patients: implications for dose optimization. Eur J Drug Metab Pharmacokinet 2020;45(6):703–13.

[31] Karatza E, Ismailos G, Marangos M, Karalis V. Optimization of hydroxychloroquine dosing scheme based on COVID-19 patients’ characteristics: a review of the literature and simulations. Xenobiotica 2021;51(2):127–38.

[32] von Rosensteil NA, Adam D. Macrolide antibacterials. Drug interactions of clinical significance. Drug Saf 1995;13(2):105–22.

[33] Jallouli M, Galcier L, Zahr N, Aumaitre O, Frances C, Le Guern V, et al. Determinants of hydroxychloroquine blood concentration variations in systemic lupus erythematosus. Arthritis Rheumatol 2015;67(8):2176–84.

[34] Chasset F, Arnaud L, Costedoat-Chalumeau N, Zahr N, Bessis D, Frances C. The effect of increasing the dose of hydroxychloroquine (HCQ) in patients with refractory cutaneous lupus erythematosus (CLE): an open-label prospective pilot study. J Am Acad Dermatol 2016;74(4):693–9.e3.

[35] Nguyen LS, Dolladille C, Drici MD, Fenoux C, Alexandre J, Mira JP, et al. Cardiovascular toxicities associated with hydroxychloroquine and azithromycin: an analysis of the World Health Organization pharmacovigilance database. Circulation 2020;142(3):303–5.

[36] Funck-Brentano C, Nguyen LS, Salem JE. Retraction and republication: cardiac toxicity of hydroxychloroquine in COVID-19. Lancet 2020;396(10245):e2–3.

[37] Gerard A, Romani S, Fresse A, Viard D, Parassol N, Granvullemi A, et al. “Off-label” use of hydroxychloroquine, azithromycin, lopinavir-ritonavir and chloroquine in COVID-19: a survey of cardiac adverse drug reactions by the French Network of Pharmacovigilance Centers. Therapie 2020;75(4):371–9.

[38] Roustit M, Guilhaumou R, Molimard M, Drici MD, Laporte S, Montastruc JL, et al. Chloroquine and hydroxychloroquine in the management of COVID-19: much kerfuffle but little evidence. Therapie 2020;75(4):363–70.

[39] Cavalcanti AB, Zampieri FG, Rosa RG, Azevedo LCP, Veiga VC, Avezum A, et al. Hydroxychloroquine with or without azithromycin in mild-to-moderate Covid-19. N Engl J Med 2020;383(21):2041–52.

[40] Skipper CP, Pastick KA, Engen NW, Bangdiwala AS, Abassi M, Lofgren SM, et al. Hydroxychloroquine in non-hospitalized adults with early COVID-19: a randomized trial. Ann Intern Med 2020;173(8):623–31.

[41] Mitja O, Corbacho-Monné M, Ubals M, Tebe C, Peñaflj J, Tobias A, et al. Hydroxychloroquine for early treatment of adults with mild Covid-19: a randomized-controlled trial. Clin Infect Dis 2020;ciaa1009, http://dx.doi.org/10.1093/cid/ciaa1009.

[42] Horby P, Mafham M, Linsell L, Staplin N, Emberson JR, et al. Effect of hydroxychloroquine in hospitalized patients with COVID-19: preliminary results from a multi-centre, randomised, controlled trial. medRxiv 2020, http://dx.doi.org/10.1101/2020.07.15.20151852, 2020.07.15.20151852. https://www.medrxiv.org/content/10.1101/2020.07.15.20151852v1. [Accessed January 25, 2021].