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CLINICAL PHARMACOLOGY

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

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 COVID-19;
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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 ($t_{1/2}$) was 102 h. These parameters in COVID-19 differed from those reported in patients with lupus, where CL/F, Vd/F and $t_{1/2}$ 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 $\geq 60\%$ (95% confidence interval: 49.5–69.0%) of COVID-19 patients.

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Abbreviations

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
σ	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 μ M (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

Table 1 Median plasma and blood pharmacokinetics parameters of hydroxychloroquine (HCQ) in several pathologies.

Plasma	COVID-19 ^f	Lupus ^a	Malaria ^d	Healthy+
V/F, L	6696	2440	2363	2851
CL/F, L/h	45.5	68.2	15.5	12.0
t1/2, h	102	19.5	106	172.3
Blood	COVID-19 ^e	Lupus ^a	RA ^c	RA ^b
V/F, L	1990	903	605	2283
CL/F, L/h	14.7	18.6	9.9	15
t1/2, h	93.8	25.9	43.3	124.3

CL/F, L/h: apparent elimination clearance, liter/hour; COVID-19: novel coronavirus disease 2019; V/F, L: apparent volume of distribution, liter; T1/2, h: half-life, hour.

^a HCQ sulfate 400 mg/day [16].

^b HCQ sulfate 400, 800, or 1200 mg/day [19].

^c HCQ sulfate 200 or 400 mg/day [20].

^d HCQ sulfate dose 800 mg then 400 mg at 6, 24, and 48 h afterward [15].

^e 96 patients (135 samples).

^f 104 patients (149 samples) in our study were assessed.

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 hospitalized 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-JOCVID).

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:

$$P_i = P_{STD} \times (COV_i / COV_{STD})^{PWR}$$

where P_{STD} is the standard value of parameter and P_i and COV_i are the parameter and covariate values of the *i*th 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, K_a (with F, as the unknown bioavailability). Given the lack of data on the absorption phase, K_a 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.

	Mean \pm standard deviation	Minimum	Maximum
Age, years	63.0 \pm 14.4	25	99
Weight, kg	79 \pm 16	40	150
Height, cm	169 \pm 11	146	192
BMI, kg/m ²	27.3 \pm 5.0	17.8	51.9
Sex, (female) %	32	NA	NA
Patient in intensive care, %	23	NA	NA
MDRD, mL/min/1.73 m ²	86.0 \pm 33.6	5	194
Creatinine, μ mol/L	98 \pm 82	34	808
Albumin, g/L	29.0 \pm 6.9	12	63
HT, %	35.0 \pm 6.5	18	49
Platelet, 10 ⁹ L	313 \pm 134	52	753
White blood cells, 10 ⁹ L	7.5 \pm 4.7	2	32.4
C-reactive protein (CRP), mg/L	86 \pm 98	2	469
ALAT, U/L	69 \pm 74	11	486
ASAT, U/L	63 \pm 49	13	252
Dose HCQ, mg/day	563 \pm 99	200	800
Observation duration, days	5.3 \pm 2.3	1	12
Blood concentration			
HCQ, ng/mL	586 \pm 457	50	2792
Plasma concentration			
HCQ, ng/mL	193 \pm 152	12	795
HCQBlood/HCQPlasma	4.0 \pm 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–Meyer 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 (C_p) as:

$$CRP = CRP_0 * \left\{ 1 - f_{HCQ} * C_p / (C_{p50} + C_p) - (1 - f_{HCQ}) * t / (t + t_{50}) \right\}$$

where CRP_0 , f_{HCQ} , C_{p50} and t_{50} denote the initial CRP concentration, fractional effect of HCQ, HCQ concentration or time that produce a 50% decrease in the CRP_0 level. The

model stands for the effect of HCQ (f_{HCQ} and C_{p50}) plus an independent time-related effect ($[1 - f_{HCQ}]$ and t_{50}), which simultaneously decrease the initial CRP_0 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.

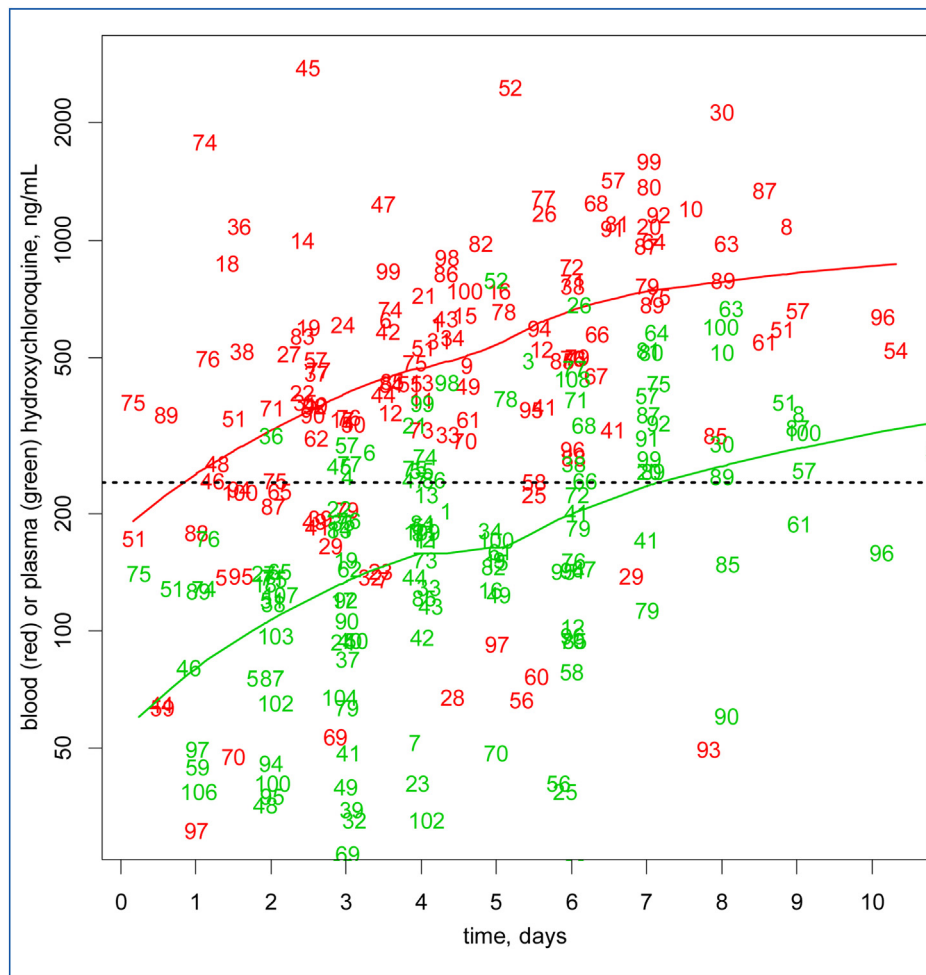


Figure 1. Observed blood (red) and plasma (green) hydroxychloroquine concentrations. Numbers stand for the patient identity and lines for the corresponding spline describing the overall trend for each matrix.

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 $\geq 40\%$ (95% confidence interval 30–50%) and $\geq 60\%$ (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](#)).

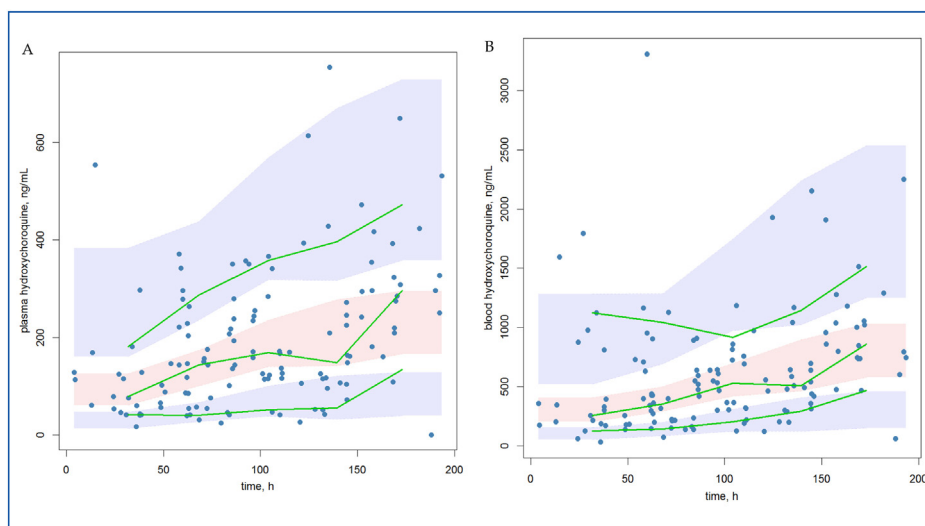


Figure 2. Prediction-corrected visual predictive check for plasma (A) and blood (B) hydroxychloroquine population pharmacokinetics. Plain (●) and green lines stand for prediction-corrected observed concentrations and their 5th, 50th and 95th percentiles. Light blue and red bands stand for the corresponding model-predicted 90% confidence intervals.

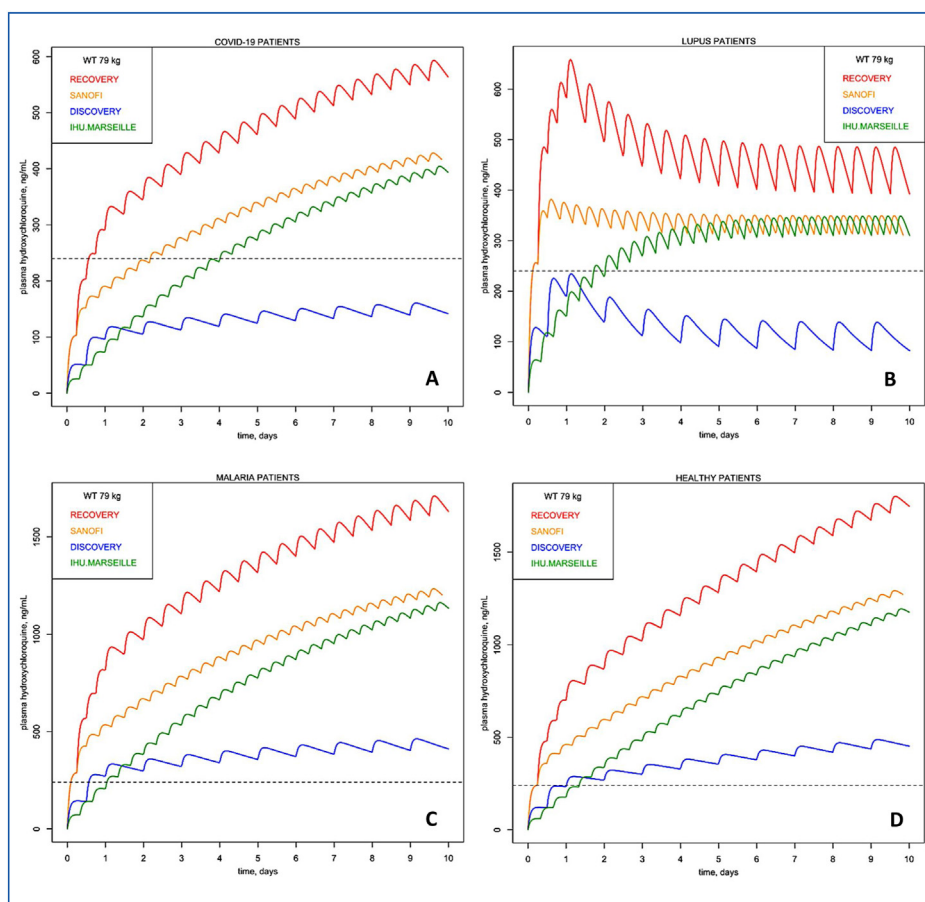


Figure 3. Representative predicted plasma hydroxychloroquine (HCQ) concentrations-time courses as a function of the dosing regimen evaluated in major prospective trials testing HCQ for COVID-19. Curves are drawn according to our final parameters for a typical patient weight (WT) of 79 kg (observed median). Dosing schedules are 2.4g 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) ^{β}	1	Fixed
CL/F	45.9	21.2
β , CL/F*(BW/70) ^{β}	0.75	Fixed
η V/F	0.61	18.9
η CL/F	0.69	25.1
σ , ng/mL	64.1	9.76

β : covariate effect parameter; η : between-subject variability; σ : 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 *i*th patient $CL/F_i = CL/F^*(BW_i/70)^{0.75}$.

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) ^{β}	1	fixed
β , V/F*(PLAT/300,000) ^{β}	-0.726	37
CL/F	14.7	13.5
β , CL/F*(BW/70) ^{β}	0.75	fixed
η V/F		
η CL/F		
σ , proportional	0.272	12.2

β : covariate effect parameter; η : between-subject variability; σ : 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 *i*th patient $CL/F_i = CL/F^*(BW_i/70)^{0.75}$ and $V/F_i = V/F^*(BW_i/70)*(PLAT_i/300,000)^{-0.726}$.

Pharmacodynamic effects of HCQ in COVID-19

A total of 75 patients were available for a SARS-CoV-2 viral status analysis using nasopharyngeal swab. PCR follow-up was negative in 40 (53%). To assess the effect of plasma HCQ concentration on time-to-PCR negatization, patients were grouped as follows: individual predicted plasma HCQ concentration at 48 h below versus above 25th (72 ng/mL), 50th (95.5 ng/mL), 75th quantile (129 ng/mL). There were no significant differences in time-to-PCR negatization for all tested comparisons (Fig. 6). In our cohort, only 4 and 16 patients among 104 had observed or imputed (in patients with data available after 72 hours)

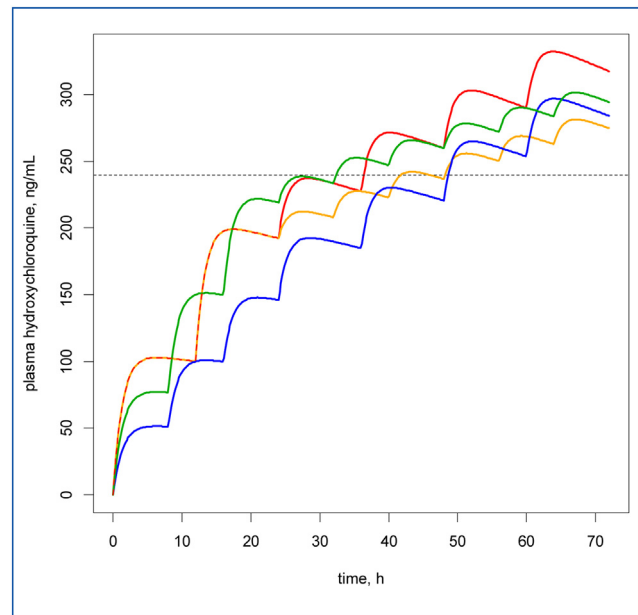


Figure 4. Possible dosing regimen in COVID-19 patients (weighing 79 kg) according to our final model. Dosing schedules represented are 800 mg/12 h (total 1600 mg) the 1st day, then 400 mg/12 h (RED); 800 mg/12 h (total 1600 mg) the 1st day, then 200 mg/8 h (ORANGE); 400 mg/8 h (total 1200 mg) loading dose the 1st day, then 400 mg/12 h (BLUE); 600 mg/8 h (total 1800 mg) loading dose the 1st day, then 200 mg/8 h (GREEN).

HCQ plasma levels >240 ng/mL, the in vitro half maximal effective concentration of HCQ against SARS-CoV-2, at 48 and 72 hours, respectively. There was also no significant effect of HCQ plasma concentration on the CRP time-course. All attempts gave non-significant values for fHCQ, or Cp50 parameters that stand for the effect of HCQ on CRP time-course, meaning that the effect of HCQ on the inflammation status could not be demonstrated.

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-

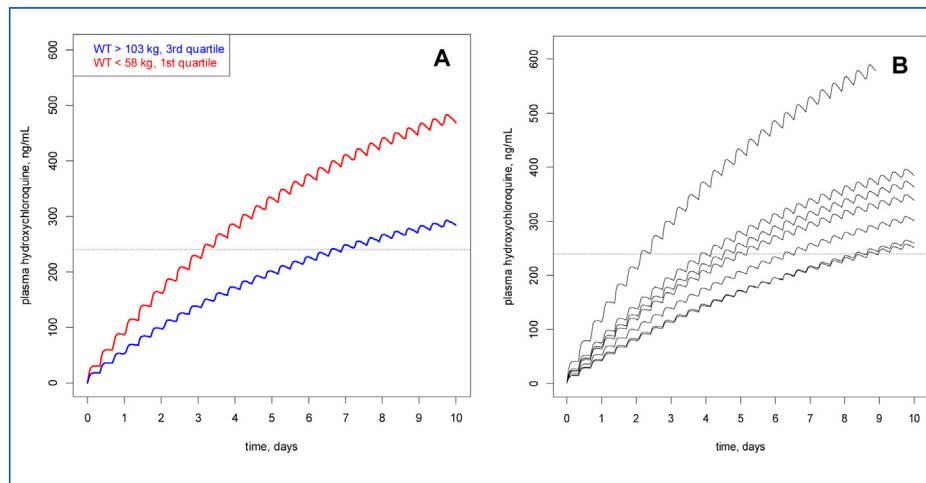


Figure 5. Mean plasma hydroxychloroquine (HCQ) concentration-time courses for a patient with bodyweight (WT) <58 kg or >103 kg and half-life >70 h, red and blue curves, respectively (A) and for typical patients with 79 kg WT and clearance (CL) ranging between 30–68 L/h and volume of distribution (Vd) 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.

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 pre-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 negatiation 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. Unfortunately, the detailed time course of viral load was unknotted available, 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

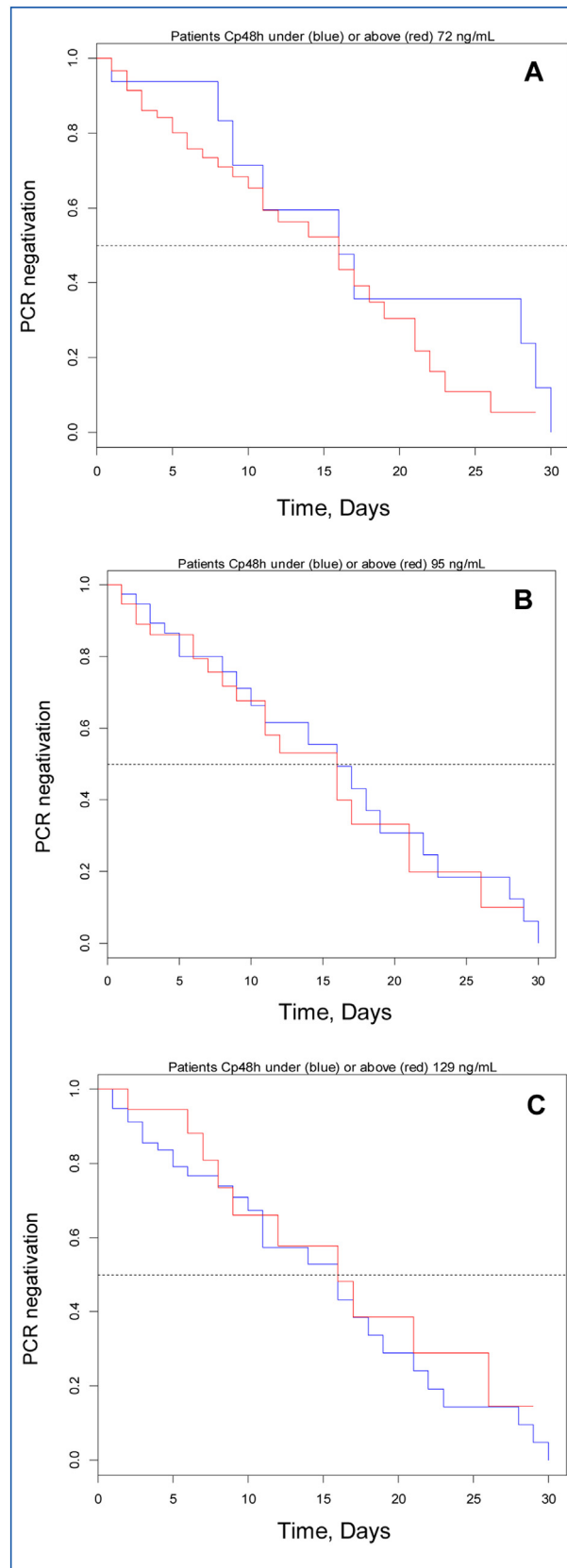


Figure 6. Time-to-Sars-Cov-2 PCR negatvation curves as a function of hydroxychloroquine (HCQ) plasma levels within 48 hours of HCQ start. Blue and red curves represent patients with an HCQ plasma concentration at 48 h below or above the 1st HCQ plasma concentration quartile observed in our cohort, respectively (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 $\geq 60\%$ (95% confidence interval: 49.5–69.0%) of COVID-19 patients.

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The authors declare that they have no competing interest.

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