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Research Article

Hydroxychloroquine: Pharmacokinetics and Toxicity

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Background/Purpose(s). We have extensively used HCQ at 200 mg three times a day (tid) to treat various infections such as Q fever and Whipple's disease. Serum levels of between 1 μg/ml and 2 μg/ml serum level are recommended to achieve the safety and efficacy of these treatments. Our aim in this paper is to describe our experience regarding the pharmacokinetics and toxicity of HCQ in another infection caused by SARS-CoV-2. *Methods*. As recommended, we performed electrocardiograms before administering HCQ off-label. The HCQ concentration in the serum was monitored to ensure the effectiveness and safety of the treatment. We retrospectively analysed HCQ serum concentrations measured over time and toxicity data in patients with COVID-19 who were treated with HCQ at the IHU Marseille Infection. We did not treat patients with HCQ contraindications with this medication. *Results*. We measured HCQ concentrations in 1310 serum samples from 989 patients with COVID-19. The mean ± SD HCQ concentration increased in patients' sera during treatment from day 1 (0.10 μg/ml ± 0.08) to day 11 (0.85 μg/ml ± 0.44), confirming that HCQ accumulates in the body during short-term therapy. However, the observed concentrations did not exceed the therapeutic range for other indications (0.80–1.20 μg/mL in Q fever patients treated for between 18 and 24 months). In patients treated with HCQ, major side effects included intestinal disorders (nausea, vomiting, and gastric pain) and QT prolongation. No conduction disorders (including torsades de pointes and ventricular arrhythmia), cardiomyopathy, retinopathy, or HCQ-related deaths were observed. *Conclusions*. In patients treated over a short time period with 200 mg tid of HCQ, therapeutic concentrations in serum were obtained in most patients without significant side effects or complications. Although patients must be carefully evaluated for HCQ contraindications, HCQ 200 mg tid for ten days can be considered an appropriate and safe dosage in patients with COVID-19.

1. Introduction

Quinine obtained from the bark of the cinchona plant (*Cinchona officinalis*), also known as "Jesuit's bark," has been used to treat malaria since the 16th century [1, 2]. This compound is an alkaloid that belongs to the arylamino alcohol group of drugs. Quinine has remained a mainstay in the treatment of severe malaria to this day [1]. Chloroquine (CQ), a synthetic drug inspired by quinine, was discovered in 1934 by Hans Andersag at the Bayer laboratories [3]. Its large-scale use as an antimalarial agent began during World War II, in the early 1940s [1, 2]. Hydroxychloroquine (HCQ) was synthesised in 1946 from chloroquine by the addition of a hydroxyl group. It was approved for medical use in 1955, as an alternative to chloroquine, due to its reduced toxicity [1, 2]. Compared to other antimalarial drugs (quinine, halofantrine, quinidine, and mefloquine), the peak plasma concentration of which is in the range of the concentration relevant for cardiac repolarisation (hERG K+ channel IC_{50}), the concentration of chloroquine active on the hERG channel is much higher (between four and 14 times the peak plasma concentration) [4].

The anti-rheumatic activity of CQ and HCQ was discovered during World War II in soldiers taking malaria prophylaxis [5, 6]. Since the 1950s, both CQ and HCQ have been used to treat patients with rheumatological disorders, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [7]. More recently, CQ and HCQ have been used in other indications. These compounds can prevent thromboses, especially in the context of

antiphospholipid syndrome [8]. They reduce the risk of cardiovascular disease in patients with RA [7] and have been used to treat neoplastic diseases [9].

With regard to infectious diseases, CQ and HCQ display broad-spectrum antimicrobial activity in vitro, including against many bacteria, fungi, and viruses [10, 11]. Due to its lower toxicity, HCQ is the preferred drug of the two [1, 2, 12]. In humans, it has been used successfully to cure bacterial diseases such as chronic Q fever [13] and Whipple's disease [14]. In Q fever endocarditis, HCQ treatment lasts for at least 18 months, with target therapeutic levels between 0.8 μg/mL and 1.2 μg/mL [15, 16]. More recently, both CQ and HCQ were found to be active in vitro against the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the RNA virus which causes coronavirus disease 19 (COVID-19) [12, 17–19]. CQ and HCQ have been extensively evaluated alone and combined with azithromycin for prophylaxis and treatment of COVID-19, with contradictory results [20–22]. Concerns have long been raised around the potential toxicity of CQ and HCQ [23–27]. The major toxic effects include cardiac toxicity (QTc prolongation, conduction disorders that may lead to torsades de pointes, ventricular arrhythmias, and cardiomyopathy) and ocular toxicity (irreversible retinopathy). Even though these molecules (particularly HCQ) have been used for decades to treat malaria, Q fever, Whipple's disease, and rheumatic diseases, a major controversy has recently arisen over the use of CQ or HCQ to treat COVID-19.

Our team reported several observational studies showing the usefulness of the combination of HCQ and azithromycin in the management of patients with COVID-19 in the early stage of infection [21, 28–30]. Patients were treated with HCQ (200 mg tid for ten days) combined with azithromycin (500 mg on the first day followed by 250 mg/day for the following four days). We have extensive experience in the use of HCQ to treat patients suffering from chronic Q fever [13] and Whipple's disease [14, 31]. In this study, our aim was to report the pharmacokinetic and toxicity data we gathered from patients with COVID-19 who we treated with HCQ.

2. Materials and Methods

2.1. Patients. Patients with COVID-19 were cared for at the Institut Hospitalier Universitaire Méditerranée Infection (IHU, Marseille, France), part of the Marseille University Hospital (AP-HM, Marseille, France). Diagnosis was based on compatible clinical and epidemiological data and a positive SARS-CoV-2 real-time PCR test upon admission, taken from a nasopharyngeal sample [32]. An oral "off-label" treatment combining HCQ (200 mg tid for ten days) and azithromycin (a single dose of 500 mg on the first day and 250 mg/day for the following four days) was proposed to patients during hospitalisation by one of the practicing physicians, independently of the investigator, after a collegial decision based on the most recent scientific data available on that date and after assessment of the benefit/harm ratio of the treatment in accordance with the provisions of the Code of Ethics (Article R. 4127-8 of the French Public Health Code). The patients were followed daily. A clinical examination was performed every day, and appropriate biological and radiological exams were prescribed when necessary. A SARS-CoV-2 real-time PCR test was performed on a nasopharyngeal sample every two days until two negative tests were obtained. We measured HCQ concentrations in 1310 serum samples from 989 patients with COVID-19, as recommended for the surveillance of therapeutic levels (1 μg/ml to 2 μg/ml) and dosage optimisation in Q fever and Whipple's disease [13, 14].

HCQ was not administrated to patients with hypokalaemia (<3.6 mmol/L); those who were taking comedications prolonging QTc other than azithromycin (assessed using https://www.crediblemeds.org/) notably citalopram, escitalopram, hydroxyzine, domperidone, and piperaquine; those with any known allergy to CQ or HCQ; those presenting severe QTc prolongation (>450 ms) on an electrocardiogram performed before treatment; or those with cardiomyopathy, severe retinopathy, or a known G6PD deficiency.

2.2. Ethics Statement. Clinical and laboratory data from patients hospitalised with COVID-19 were recorded as part of routine care in the hospital's electronic health recording system (AXIGATE®). The data used for this study were extracted retrospectively from the database. The processing of personal data followed the MR-004 reference methodology and was registered under No. RGPD APHM 2020-152. Data accessibility is protected in line with the European General Data Protection Regulation No. 2016/679. The retrospective nature of the study was approved by our institutional review board committee (Méditerranée Infection No. 2020-13).

2.3. Measurement of HCQ Concentrations in Serum. Native HCQ concentrations were measured in patients' serum samples by UHPLC-UV using a previously described protocol [33]. The peak of the chromatogram at a retention time of 1.05 minutes corresponds to HCQ metabolites. The serum concentrations of HCQ and its metabolites (bidesethylchloroquine, desethyl hydroxychloroquine, and desethylchloroquine) were deduced from UV absorption. The consideration of both concentrations provided an estimate of the initial serum HCQ concentration.

As part of routine surveillance, HCQ ophthalmic toxicity was evaluated by daily examination and, if necessary, final visual acuity (FVA) and Spectral Domain Optical Coherence Tomography (SD-OCT). Cardiac toxicity was surveyed at baseline and a few days after the start of HCQ treatment by cardiac electrocardiography (ECG), particularly to check for elongated QTc intervals. Skin toxicity was evaluated daily by a skin exam. Kidney toxicity was evaluated by measuring serum creatinine levels every two days.

2.4. Statistics. Statistical tests were performed using StatPlus (AnalystSoft, Walnut, CA, USA).

3. Results

3.1. Population Demographics and Clinical Symptoms. The demographics of the COVID-19 patients included in this study are summarised in Table 1. These patients included 546 women and 443 men, with a median age of 50 and 52 years, respectively. Chronic comorbidities included hypertension (198 patients, 20.02%), diabetes (110 patients, 11.12%), obesity (108 patients, 10.92%), chronic respiratory diseases (103 patients, 10.41%), chronic heart diseases (81 patients, 8.19%), and cancer (51 patients, 5.16%). Patients presented with fever (199 patients, 20.12%), cough (420 patients, 42.47%), rhinitis (183 patients, 18.5%), anosmia (183 patients, 18.5%), ageusia (170 patients, 17.19%), dyspnoea (201 patients, 20.32%), and thoracic pain (109 patients, 11.02%). A pulmonary CT scan was performed on 737 of the 989 patients (74.52%). The CT scan was normal in 208 patients (28.22%), while others revealed minimal lung lesions (308 patients, 41.79%), intermediate lung lesions (162 patients, 21.98%), or severe lung lesions (59 patients, 8.01%).

3.2. HCQ Serum Concentration in COVID-19 Patients. Serum concentrations of HCQ over time in COVID-19 patients are presented in Figure 1 and Table S1. On day 1 of HCQ and azithromycin administration, the mean ± SD and median HCQ concentrations, as determined for 56 patients, were 0.10 μg/mL ± 0.08 μg/mL (range: 0–0.31) and 0.09 μg/mL, respectively. On day 2, for 297 patients, they were 0.20 μg/mL ± 0.13 μg/mL (range: 0–1.27) and 0.17 μg/mL. On day 3, for 124 patients, they were 0.23 μg/mL ± 0.16 μg/mL (range: 0–0.98) and 0.21 μg/mL. The mean serum concentration then increased from 0.38 μg/mL ± 0.21 μg/mL on day 4 to 0.85 μg/mL ± 0.44 μg/mL on day 11, the day following discontinuation of HCQ treatment. Serum concentrations then decreased progressively as shown in Figure 1 and Table S1. Interestingly, for 9/297 patients (3%), we did not detect any HCQ in their serum on day 2, and only a few HCQ measurements were positive over the following days (Table S2). Furthermore, for 55/299 patients (18.4%), the HCQ concentration on day 2 was lower than 0.1 μg/mL (range: 0.016–0.099), which is considered the likely minimal therapeutic HCQ concentration according to the guidelines established in March/April 2020 by the French National AC43-ANRS/STP-SFPT Team and according to our previous publication on 1061 patients [10, 12].

3.3. Parameters Influencing HCQ Serum Concentrations in COVID-19 Patients. We first examined the parameters that could influence the serum concentration of HCQ on day 2 due to the availability of this measurement in 297 patients (Table 2). HCQ concentration was negatively correlated with age on day 2 ($n = 299$, Rho −0.21, $P < 0.001$) and day 4 ($n = 124$, Rho −0.18, $P = 0.045$). Patients under the age of 65 ($n = 227$, mean ± SD: 0.20 μg/mL ± 0.11, median: 0.18 μg/mL) had significantly higher concentrations on day 2 than patients over the age of 64 ($n = 70$, mean ± SD: 0.17 μg/mL ± 0.18, median: 0.11 μg/mL, $P = 0.0001$ (Kruskal–Wallis), $P < 0.01$ (chi-square test)).

We found that women had significantly higher HCQ serum concentrations than men (women, $n = 159$, HCQ serum concentration mean ± SD: 0.22 μg/mL 0.15, and median: 0.20 μg/mL; men, $n = 138$, HCQ serum concentration mean ± SD: 0.17 μg/mL ± 0.10, and median: 0.15 μg/mL; $P < 0.001$ (Kruskal–Wallis) and $P < 0.01$ (chi-square test)). On day 3, in 124 patients, women still had higher concentrations than men (women, $n = 65$, mean ± SD: 0.27 μg/mL ± 0.19, median: 0.24 μg/mL compared to men, $n = 62$, mean ± SD: 0.20 μg/mL ± 0.12, median: 0.18 μg/mL; $P = 0.034$ (Kruskal–Wallis) and $P = 0.0163$ (chi-square test)). HCQ serum concentrations were also found to be significantly associated with sex on day 4 ($n = 124$, $P < 0.001$), day 6 ($n = 60$; $P = 0.039$), and day 10 ($n = 136$; $P = 0.0001$).

Body mass index (BMI) information was available for 186/989 patients (Table 1). BMI was negatively associated with HCQ serum concentration on day 2 ($n = 39$, Rho −0.36, $P < 0.022$, Rho −0.35) and on day 4 ($n = 16$, Rho −0.58, $P = 0.017$, Rho −0.57) (data not shown), but obesity (BMI > 30) as well as comedications, including in patients receiving eight or more drugs (other than HCQ and azithromycin), was not significantly associated with HCQ concentration (Table 2). The higher the number of comorbidities, the lower the HCQ concentration.

3.4. HCQ Toxicity. Major side effects in COVID-19 patients treated with HCQ included intestinal disorders (nausea, vomiting, and gastric pain), QT prolongation over 500 ms, and an increase in QTc of more than 60 ms in 20 patients (2.02%), respectively. No patients treated with HCQ experienced conduction disorders such as torsades de pointes or ventricular arrhythmia. No sudden deaths related to HCQ administration were reported. No cardiomyopathy or cardiac failure developed during HCQ treatment. Finally, no retinopathy was detected by clinical exam or ophthalmological surveillance. As seen in Figure 1, three patients were extreme outliers for HCQ serum dosage (Table 3). No drug-related toxicities were found in any of these extreme outliers. In addition, the 20 patients who experienced a QTc prolongation under the HCQ-plus-azithromycin protocol presented no statistical differences ($P = 0.79$, Wilcoxon test) in HCQ serum concentrations compared to patients without QTc prolongation (Table 4).

Of the 989 COVID-19 patients who participated in this study, an unfavourable evolution after hospitalisation was observed in 132 patients (13.35%), a hospital stay longer than 10 days in 120 patients (12.13%), a need for ICU admission in 34 patients (3.44%), and death in 18 patients (1.82%) (Table 5). Patients with a poor outcome, i.e., those who experienced a long hospital stay (≥10 days), ICU admission, or death, did not display significant differences ($P = 0.68$, Wilcoxon test) in HCQ serum concentrations compared to patients without these severe disease markers (Table 6).

Table 1: Patient demographics.

COVID-19 patients	Women			Men			Total		
	n	Mean (min-max)	Median	*n*	Mean (min-max)	Median	*n*	Mean (min-max)	Median
Age (years)	546	49.3 (18–96)	50	443	50.9 (18–98)	52	989	50.0 (18–98)	50
Body mass index	102	28.7 (17–52)	28.8	84	28.8 (20–52)	28.3	186	28.8 (17–52)	28.7
Number of comedications	423	1.4 (0–15)		370	1.5 (0–18)		793	1.4 (0–18)	

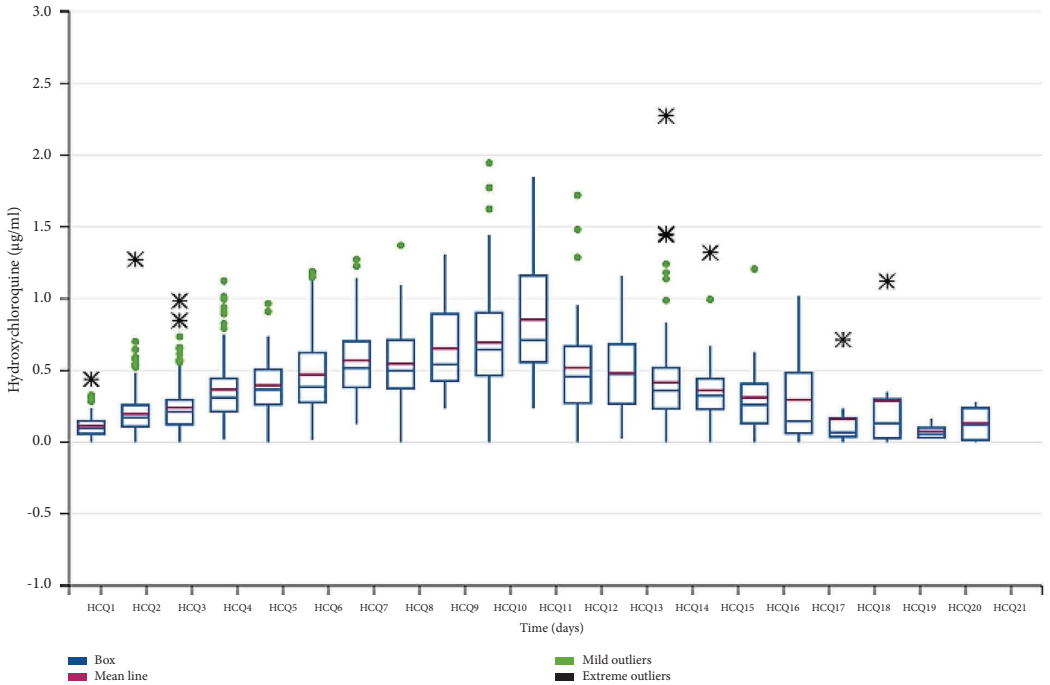

Figure 1: Serum concentrations of HCQ over time. Serum concentration of HCQ at different time points (from day 0 to day 28) after the start of treatment with HCQ 200 mg tid combined with azithromycin for ten days in 989 patients (1310 dosages).

Table 2: HCQ serum concentrations two days after treatment onset according to age, sex, comedications, and comorbidities.

	HCQ serum concentration (μg/mL) two days after treatment onset		
	n	Mean (SD)	*P* (Kruskal–Wallis test)
Age (years)			
>64	70	0.17 (0.18)	0.0001
55–64	68	0.19 (0.11)	
45–54	53	0.23 (0.11)	
18–44	106	0.20 (0.12)	
Sex			
Women	159	0.22 (0.15)	0.0009
Men	138	0.17 (0.10)	
Body mass index			
<30	20	0.17 (0.08)	0.0637
>30	19	0.12 (0.08)	
Comedications (≥5 drugs)			
No	95	0.21 (0.12)	0.129
Yes	183	0.19 (0.14)	
Comedications (≥8 drugs)			
No	274	0.20 (0.13)	0.539
Yes	4	0.24 (0.17)	
Number of comorbidities			
0	169	0.21 (0.11)	0.0002
1	71	0.20 (0.17)	
2	40	0.15 (0.13)	

Table 3: Main characteristics of extreme outliers for COVID-19 patients.

Patient	Sex	Day	Age (years)	HCQ serum level (μg/mL)	Clinical outcome	Drug-related adverse event	Death
1	F	2	65	0.804	Good	No	No
7	F	16*	37	1.452	Good	No	No
9	F	17$	89	1.32	Poor	No	Yes

*Patient already taking HCQ before COVID-19 for lupus. $Treatment duration with HCQ was extended due to persistence of viral shedding and acute respiratory distress.

Table 4: HCQ concentrations in serum over time for COVID-19 patients with or without QTc prolongation.

Days		1	2	4	6	8	9	12
QTc [34]	n	20	20	20	20	20	20	20
	(HCQ)	0.13 ± 0.04	0.29 ± 0.19	0.22 ± 0.07	0.36 ± 0.25	0.59 ± 0.21	0.90 ± 0.19	0.29 ± 0.09
No QTc	n	56	297	127	64	41	20	40
	(HCQ)	0.10 ± 0.09	0.20 ± 0.13	0.38 ± 0.22	0.48 ± 0.30	0.57 ± 0.29	0.65 ± 0.30	0.52 ± 0.37

QTc: QTc prolongation; no QTc: no QTc prolongation. n: number of patients tested at each time point. (HCQ): HCQ concentrations over time (μg/mL, mean ± SD).

Table 5: Clinical outcome.

Clinical outcome	n patients	% of patients
Unfavourable evolution	132	13.35
Hospitalisation ≥10 days	120	12.13
ICU admission	34	3.44
Death	18	1.82

Table 6: HCQ concentration in serum according to clinical outcome.

Days		2	4	6	8	10	12	14
Good outcome	n	255	96	33	17	118	35	109
	(HCQ)	0.19 ± 0.12	0.38 ± 0.23	0.51 ± 0.29	0.43 ± 0.19	0.67 ± 0.32	0.518 ± 0.38	0.42 ± 0.33*3
Poor outcome	n	42	28	27	22	18	5	4
	(HCQ)	0.18 ± 0.21	0.32 ± 0.18	0.42 ± 0.29	0.64 ± 0.31	0.86 ± 0.52	0.59 ± 0.25	0.34 ± 0.22

"Poor outcome" means hospitalisation ≥10 days, ICU admission, or death. "Good outcome" means none of the severity markers of these three outcomes. n: number of tested patients at each time point. (HCQ): HCQ concentrations over time (μg/mL, mean ± SD).

4. Discussion

Over the past 30 years, our team used long-term treatments combining HCQ with antibiotics to treat chronic life-threatening diseases such as chronic Q fever [13] and Whipple's disease [14, 31, 35]. For both diseases, HCQ has been administrated orally at a dosage of 200 mg tid over several months. In our experience, HCQ is effective at a serum concentration of approximately 1 μg/ml [12]. Measurements of HCQ concentrations were performed throughout the treatment to monitor efficacy and toxicity.

More recently, HCQ has been proposed for the prevention and treatment of COVID-19. This recommendation is based on the in vitro activity of HCQ on SARS-CoV-2 [12, 17–19]. Several studies on COVID-19 patients have confirmed the prophylactic and therapeutic benefit of HCQ [28, 29, 34, 36]. HCQ has also been reported as being effective at reducing the length of hospital stays and the need for ICU admission [37, 38]. HCQ has several potential mechanisms of action [39]. It can alkalinise the acid compartments of eukaryotic cells preventing virus endocytosis and replication [18, 40]. HCQ displays anti-inflammatory properties by inhibiting the production of the proinflammatory cytokines interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), IL-1-β by activated macrophages, and the production of chemotactic cytokines involved in the recruitment of proinflammatory cells in the lungs [41–43]. Most of these inflammatory markers are associated with COVID-19 severity [44, 45].

In addition, two major studies have shown that chloroquine interacts with both the sigma-1 and sigma-2 receptors, which bind the Nsp-6 and ORF9c proteins of SARS-CoV-2, respectively [46, 47]. These receptors modulate the endoplasmic reticulum stress used by the virus to promote its multiplication [48]. The importance of these receptors in infection is confirmed by the in vitro anti-SARS-CoV-2 efficacy of several specific ligands for both sigma-1 and sigma-2 receptors [48].

According to our experience with the use of HCQ, we proposed the oral administration of 200 mg tid of HCQ for patients with COVID-19, either alone or later combined with azithromycin [28, 30]. Azithromycin is active against SARS-CoV-2, other viruses, and some bacterial species and displays anti-inflammatory properties [18, 49]. Because we suspected that HCQ could block viral replication at the early stage of COVID-19 in vivo, HCQ and azithromycin were administered on the day of the RT-PCR confirmation of SARS-CoV-2 infection and for ten and five days, respectively.

We first assessed the parameters influencing HCQ serum concentrations in COVID-19 patients. On day 2 of HCQ treatment, women had significantly higher concentrations than men (Table 2; 0.20 μg/mL versus 0.15 μg/mL median HCQ concentration). Women still had a significantly higher HCQ serum concentration on days 3, 4, 6, and 10. Several drugs have sex-related pharmacokinetics. This is a multifactorial phenomenon [50]. Among these factors, the secretion of gastric fluids differs between women and men. Basal and maximal flow of gastric fluid and acid secretion is higher in men than women, resulting in an increased absorption of weak bases such as antimalarial drugs. Another explanation may be the distribution of the drug, which is usually greater in men than in women and resulting, for the same dose of drug, in increased serum levels in women [51]. The absorption rate of a drug is also influenced by transit times and gut motility. Transit times differ significantly between men and women, being shorter in men than in women. Some of the hepatic CYP450 enzymes clearly show sex-related differences [50]. This is the case of CYP450 2D6 which increases the levels of some drugs in women such as codeine and flecainide. It is interesting to note that HCQ is metabolised by CYP450 P2D6, another possible explanation for our findings [52].

BMI information, available for 186/989 patients, was negatively associated with HCQ serum concentration on day 2 and on day 4. HCQ concentration was also negatively correlated with age on day 2 and day 4 (Table 2). It could be hypothesised that the same phenomenon of distribution volume related to BMI may be occurring. The larger the BMI, the lower the HCQ level.

On day 2 of HCQ administration, significantly higher concentrations were found in patients under the age of 65 ($n = 227$) than in those over 64 ($n = 70$), with a median HCQ concentration of 0.18 μg/mL versus 0.11 μg/mL, respectively (Table 2). Age-related drug pharmacokinetics might be linked to age-related alterations in intestinal or hepatic CYP3A4 activity. This has been shown for erythromycin clearance between pre- and postmenopausal women [50].

The pharmacokinetic parameters of HCQ have been previously defined [39, 53, 54]. When administered orally as a single dose, the gastrointestinal absorption of HCQ is fast and its blood concentration culminates after two to three hours. The bioavailability of HCQ is 70%–80% [39, 53, 54]. HCQ can accumulate in most tissues (including the lungs), where residual concentrations can be detected for several months [39, 53, 54]. The large volume of distribution and the long half-life of HCQ are likely to be related to its accumulation in eukaryotic cell acidic compartments [39, 53, 54]. As indicated above, HCQ is transformed into deacetylated metabolites in the liver by cytochrome P450 enzymes CYP3A4, CYP2D6, CYP2C8, and CYP 3A5 [39, 53, 54]. It is mainly eliminated by the kidney and, to a lesser extent, via faeces and the skin [39, 53, 54]. Few clinical studies have published serum concentrations of HCQ. Most RCTs have been conducted using different HCQ dosages and some, such as RECOVERY, using doses that are considered as toxic (2400 mg as loading dose). Most of the time, data on HCQ concentrations are not comparable. Some studies on smaller samples showed that our mean HCQ serum was close to those found in plasma and adapted to levels needed to inhibit SARS growth in vitro [18]. Overall, HCQ plasma or serum concentrations may vary according to the patient's compliance, intestinal absorption, bioavailability, drug interactions, liver metabolism, and kidney elimination [39, 53, 54].

It has been hypothesised that taking proton pump inhibitors (PPIs) with HCQ may reduce the immunomodulatory effects of HCQ [55]. Both PPI and HCQ are weak bases that accumulate in acidic cell environments, which might lead to antagonistic effects [55]. In contrast, no deleterious effect of PPI on HCQ bioavailability was observed [56]. Drug-drug interactions may occur between HCQ and all other drugs metabolised by the above cytochrome P450 enzymes [53, 57]. As examples, HCQ can increase the plasma concentration of digitoxin, metoprolol, and dextromethorphan [53].

Yao et al. [58] determined the HCQ half-maximal effective concentration (EC50), i.e., the concentration of HCQ required to obtain 50% of the maximum SARS-CoV-2 inhibitory activity of this molecule at 0.72 μM (i.e., 0.242 μg/mL) after 48 hours of exposure. They estimated that for chloroquine, concentrations in the lung tissue could reach 400 times that of the plasma [58]. In a different model, Liu et al. [12] reported EC50 at 48 hours varying from 4.51 μM to 12.96 μM (i.e., 1.51 μg/ml and 4.35 μg/ml, respectively) according to the multiplicity of infection (MOIs; from 0.01 to 0.8). Our team demonstrated a strong synergistic effect in vitro of the combination of HCQ with azithromycin against SARS-CoV-2, with an HCQ concentration required for clearance (EC99) of SARS-CoV-2 of 5 μM (1.679 μg/mL) [18]. According to Yao et al. [58], all the above HCQ concentrations are achievable in pulmonary tissue. In the present study, we evaluated the pharmacokinetics of HCQ serum concentrations in a cohort of 989 COVID-19 patients, including the time required to obtain therapeutic concentrations. We also assessed the parameters influencing HCQ concentrations over time. In COVID-19 patients tested two days after treatment ($n = 297$ patients), the median serum HCQ concentration was 0.17 μg/mL. It was 0.32 μg/mL on day 4 ($n = 127$), 0.44 μg/mL on day 6 ($n = 64$), 0.57 μg/mL on day 8 ($n = 41$), 0.65 μg/mL on day 10 ($n = 138$), and peaked at 0.71 μg/mL on day 11 ($n = 15$) (Figure 1, Table S1). Then, HCQ serum concentrations declined, although a median concentration of 0.26 μg/ml was still observed on day 16 ($n = 20$), i.e., six days after treatment withdrawal. HCQ serum concentrations were above 0.2 μg/ml from day 3 to

Table 7: In vitro susceptibility to hydroxychloroquine of SARS-CoV-2 grown in African green monkey kidney Vero E6 cells.

Ref.	SARS-CoV-2 strain tested	HCQ concentrations tested, time of treatment administration, MOI[$]	Antiviral effect evaluation method	Results	EC50 and EC90 converted from μM to μg/ml
[79]	IHUMI-3	0.1–100 μM, 4 h before viral infection, MOI = 0.25	Inhibition of viral replication (RT-PCR quantification) 48 h p.i.	EC50 = 1.5 ± 0.3 μM EC90[£] = 3.0 ± 1.9 μM	EC50 = 0.65 ± 0.13 EC90 = 1.29 ± 0.82
[18]	IHUMI-3	HCQ 5 μM + azithromycin 5 μM or 10 μM, 4 h before viral infection, MOI = 0.25	Inhibition of viral replication (RT-PCR quantification) 60 h p.i.	97.5% and 99.1% viral inhibition, respectively	
[80]	hCoV-19/Italy/UniSR1/2020	0.1–10 μM, 1 h before viral infection	Inhibition of virus CEP, 72 h p.i. Inhibition of viral replication (RT-PCR quantification), 72 h p.i.	EC50[£] = 4.42 μM EC50 = 4.99 μM	EC50 = 1.9 EC50 = 2.15
[81]	BetaCoV/Korea/SNU01/2020	1 or 2 μg/ml, 1 h p.i., MOI = 0.05	Inhibition of viral replication (RT-PCR quantification), 24 h and 48 p.i.	No effect compared to growth control	
[58]	C Tan nCoV Wuhan strain 01	0.032–100 μM, 2 h p.i., MOI = 0.01	Inhibition of viral replication (RT-PCR quantification), 24 h and 48 h p.i.	EC50 = 6.14 μM and 0.72 μM, at 24 h and 48 h exposure	EC50 = 2.64 and 0.31 at 24 h and 48 h
[82]	Human strain isolated at Universitas Airlangga Hospital, Surabaya, Indonesia	0.2–400 μg/mL, 48 h p.i., MOI = 0.04	Inhibition of viral replication (RT-PCR quantification), 24 h, 48 h and 72 h p.i.	EC50 = 9.5 μg/ml, 4.7 μg/ml, and 1.4 μg/ml at 24 h, 48 h, and 72 h p.i. No viral production at 37.5 μg/ml	
[83]	Provided by Egyptian Army	1.1 ± 0.13 μg/ml, 2 h p.i., MOI = 0.1	Inhibition of viral replication (RT-PCR quantification), 72 h p.i.	EC50 = 0.385 ± 0.01 μg/ml	
[84]	MT121215.1	2–5 μM of RAC-HCQ (same as HCQ), R-HCQ or S-HCQ sulphate[&], 1 h before infection	Inhibition of viral replication (RT-PCR quantification), 48 h p.i.	EC50 = 5.09, 3.05, and 5.38 μM, for RAC-HCQ, R-HCQ, and S-HCQ sulphate, respectively	EC50 = 2.19, 1.31 and 2.31
[85]	USA-WA1/2020	1–50 μM, 1 h before infection	Inhibition of viral CPE, 48 h p.i. Inhibition of viral RNA transcription (RT-PCR quantification), 48 h p.i. Inhibition of production of infectious progeny viruses (TCID method[#]), 48 h p.i.	EC50 = 16.5 μM EC90 = 23 μM EC50 = 18.6 μM EC90 = 25.1 μM EC50 = 21.7 μM EC90 = 24.3 μM	EC50 = 7.09 EC90 = 9.89 EC50 = 7.99 EC90 = 10.8 EC50 = 9.33 EC90 = 10.45

p.i.: post-infection; PFU: plaque forming units; CEP: cytopathic effect. [$]MOI: multiplicity of infection, which represents the ratio of the number of virus particles to the number of the host cells at the time of cell infection. [£]EC50 and EC90: drug concentration allowing 50% and 90% of the assessed effect, respectively (e.g., inhibition of viral cytopathic effect or reduction in viral load). [&]Rac-HCQ, racemic hydroxychloroquine; R-HCQ, right-handed enantiomer of HCQ; S-HCQ, left-handed enantiomer of HCQ. [#]TCID50 or TCID90: amount of virus dilution required to induce cytopathic effects in 50% or 90% of wells containing the inoculated cell culture after a defined period.

day 16 after starting administration of this drug. Loading doses, which are often advocated, have never been proven to be efficient and are likely to increase the relative risk of toxicity. For this reason, the earlier the treatment is introduced, the better the outcome is. On day 26 (16 days after stopping treatment), HCQ was undetectable, which correlates well with our understanding of the long half-life of this drug [59]. Interestingly, for 9/297 patients (3%) tested on day two following treatment prescription, we did not detect HCQ in the patient's serum, and only a few HCQ measurements were positive over the following days (Table S2). Furthermore, for 55/297 patients (18.5%), the HCQ concentration was <0.1 μg/mL on day 2 (range: 0.016 μg/mL to 0.099 μg/mL), which could be considered lower than the minimal therapeutic concentration [28]. These low HCQ serum concentrations could correspond to noncompliance with treatment by the patients (either no drug intake or delayed drug intake), low digestive absorption and distribution of HCQ, or both. According to interviews with the patients, we estimated that the nonobservance rate was close to 2.5%.

In our cohort of COVID-19 patients receiving HCQ, the most frequently observed side effects included intestinal disorders (nausea, vomiting, and gastric pain). QT prolongation over 500 ms and an increase in QTc of more than 60 ms were observed in 20 (2.02%) patients. However, no major complications occurred, including conduction disorders such as torsades de pointes or ventricular arrhythmia, HCQ-related sudden death, cardiomyopathy, cardiac failure, or retinopathy. No specific drug toxicity was found in the three patients with the highest HCQ serum concentrations. HCQ concentrations were not statistically different in the 20 patients who experienced a QTc prolongation under the HCQ-plus-azithromycin treatment compared with patients without QTc prolongation.

Common side effects of CQ and HCQ include gastrointestinal tract disorders (nausea, vomiting, and diarrhoea) and skin toxicity (skin rash, pruritus, and hair loss) [39, 60]. A more severe skin manifestation is referred to as generalised pustular figurate erythema [61]. Rare complications include myopathy, severe hypoglycaemia, haematological disorders, renal failure, and allergic reactions [24, 39, 60, 62–67]. HCQ can cross the placenta [53, 60]. Huybrechts et al. [68] recently reported a small increase in the risk of foetal malformations in pregnant women treated with HCQ during their first trimester of gestation for autoimmune rheumatic disorders. Neurological toxicity has been rarely reported [39, 60].

The most severe toxicity related to CQ and HCQ is retinopathy in patients receiving long-term therapy (e.g., for inflammatory disease) [39, 60]. These drugs cause lipofuscin accumulation in the retinal pigment epithelium, leading to a circular defect known as "bull's eye maculopathy." This retinopathy can progress for several months, even after discontinuation of treatment. A retinopathy prevalence of 7.5% was reported in patients with rheumatic disease treated with variable doses of HCQ for at least five years [69]. This prevalence was less than 2% in patients receiving between 4 mg/kg and 5 mg/kg HCQ for ten years [69]. Although higher HCQ concentrations are administrated in COVID-19 patients, retinopathy is unlikely to occur due to the short duration of treatment [69, 70].

Another severe side effect is cardiac toxicity leading to QT prolongation, rhythm disorders, cardiomyopathy, and heart failure [39, 60]. Being of old age, long-term use of CQ or HCQ treatment, use of high doses, preexisting heart conditions, and renal failure are risk factors for cardiotoxicity [39, 60]. Goldman et al. [71] analysed data (2014–2019) from the US Food and Drug Administration Adverse Events Reporting System (FAERS) database for cardiovascular events related to the use of HCQ and CQ. Among 6 677 225 reports contained in the entire database, 4895 reports (0.073%) were adverse events related to CQ or HCQ, including 696 (14.2%) cardiovascular events. These complications mainly occurred in patients taking long-term CQ or HCQ treatment for systemic lupus erythematosus or Sjogren's syndrome. Major events included cardiomyopathy (1.8%), QT prolongation (0.9%), cardiac arrhythmias (2.4%), and heart failure (2.8%). In these patients, a mortality rate of 8% was reported. Papazisis et al. [72] reported a similar study for the 2004–2019 period. Considering global side effects, including cardiomyopathy and cardiac arrhythmias, HCQ was found to be much safer than CQ. Although cardiac arrhythmias are rare events, patients should be evaluated on a regular basis, including by clinical, biological, and electrocardiogram investigations. As for COVID-19 treatment, initial cardiac arrhythmias and predisposing factors for this complication should be evaluated before administering HCQ treatment. HCQ should be avoided in patients with a QTc over 500 ms or an increase in basal QTc over 60 ms [73]. It should be administered with caution in patients taking other drugs that prolong the QT interval. In a retrospective study of the World Health Organization's pharmacovigilance database, azithromycin monotherapy was associated with a greater reporting of prolonged-QT intervals and/or torsade de pointe-associated ventricular tachycardia than hydroxychloroquine monotherapy (736/89 085 (0.8%) versus 263/76 215 (0.3%), respectively; reporting odds ratio, 2.36 [95% CI, 2.05–2.71]) [74]. The combination of azithromycin and hydroxychloroquine was associated with a greater reporting of prolonged-QT intervals and/or torsade de pointe-associated ventricular tachycardia reporting than either drug in monotherapy (999/165 300 (0.6%) versus 9/607 (1.5%), reporting odds ratio, 2.48 [95% CI, 1.28–4.79]). HCQ is considered one of the safest drugs available to treat autoimmune diseases, despite long-term administration, including in patients with systemic lupus erythematosus, RA, primary Sjogren's syndrome, antiphospholipid syndrome, and sarcoidosis [5, 7, 9, 75]. HCQ is usually administered orally at 200 mg/day to 400 mg/day, with a maximum dose of 5 mg/kg/day, and is well tolerated at this dosage [5, 7, 9, 75]. Our experience of HCQ is that, with simple precautions around use (hypokalaemia and a normal ECG), HCQ is an extremely safe drug [76]. In the long term, the most frequently reported adverse effect is retinal toxicity, which occurs only with prolonged treatment. Consequently, we felt very comfortable offering a potentially efficacious (based upon in vitro

activity) and safe solution to an emerging disease. While science is always open to debate, our compiled data on more than 3θ 000 patients confirmed our primary findings. In our sample population, HCQ-AZ was consistently associated with the lowest mortality [77]. While the misuse of HCQ in renowned trials might obviously have an impact on the outcomes of patients, the discrepancies between studies on the therapeutic effect of HCQ on COVID-19 are also likely to be due to the methodology used in clinical trials. RCTs were often underpowered and inappropriately conclusive [77].

In our work, the administration of hydroxychloroquine has never been left to chance. For some 30 years, it has been the basis of treatment for chronic Q fever and Whipple's disease, at a dosage of 600 mg per day, prescribed for one or two years, without ever having had any ocular or cardiovascular accidents. The only elements reported were occasional digestive disorders and/or muscle pain. The tolerance of hydroxychloroquine in rheumatic infections is also excellent [78]. We had performed more than 4000 HCQ dosages in the three years preceding the COVID-19 epidemic, in patients receiving long-term treatment, starting within the first days of the start of treatment. During this work, we saw a progressive increase in the concentration of HCQ, up to 1 μg/ml after 11 days. At the same time, many studies, including those published by our team, have tested the efficacy of HCQ in vitro (Table 7). The fact that microbiologists use concentrations in μg/ml and virologists use concentrations in μmol/ml may have led to confusion. In practice, 1 μg/ml corresponds to 2.34 μmol of HCQ sulphate. The studies carried out initially showed that a concentration of 1 μmol/ml inhibited the growth of the first SARS-2 strains tested. Numerous studies using different protocols and concentrations have confirmed the in vitro efficacy of HCQ, the mechanisms of which have been studied relatively extensively. In addition, HCQ is used for its anti-inflammatory effects, which we soon came to believe played a role in the late stages of SARS-2 infection. We usually consider the ratio of serum concentration to the minimum inhibitory concentration for anti-infectives, which for HCQ was 2.5, a ratio that can be considered favourable as HCQ concentrates in cells and particularly in the lung. Higher doses, such as 2.4 g on the first day used in the "Recovery" study, should be avoided, as they are toxic doses. Lower doses, such as 400 mg per day used in the "Solidarity" study, are not the doses we are used to working with because they are associated with serum concentrations which are too low [78]. Under these conditions, we proposed the use of HCQ in the way that we knew it was safe, which allowed us to have a biological activity compatible with the known data of intracellular concentration, with a satisfactory ratio of serum/MIC concentrations. HCQ administered at 200 mg tid for ten days, with or without azithromycin, can be considered a safe and effective treatment for COVID-19 patients.

Data Availability

The data used to support the findings of this study are available from the first author upon request without restriction.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Table S1: statistics of HCQ serum concentrations in COVID-19 patients over time. Table S2: HCQ serum concentrations for COVID-19 patients testing negative on day 2. (*Supplementary Materials*)

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