Hydroxychloroquine lung pharmacokinetics in critically ill patients with COVID-19

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Running title: Hydroxychloroquine concentration in lung epithelial lining fluid of COVID-19 patients.

Abstract

Different dosage regimens of hydroxychloroquine were used to manage COVID-19 patients, with no information on the lungs' exposure in this population. The aim of our study was to evaluate hydroxychloroquine concentrations in the lung epithelial lining fluid (ELF) in patients infected with COVID-19. This study is a retrospective, observational, multicenter, pharmacokinetics study of hydroxychloroquine in critically ill patients. No additional interventions or additional samples compared to standard care of these patients were conducted in our teaching hospital. We included all intubated COVID-19 patients treated with crushed hydroxychloroquine tablets, regardless of the dosage administered by the nasogastric tube. Blood and bronchoalveolar lavage (BAL) samples (n= 28) were collected from 22 COVID-19 patients and the total hydroxychloroquine concentrations in epithelial lining fluid were estimated. Median hydroxychloroquine plasma concentrations were of 0.09 [0.06; 0.14] mg/l and 0.07 [0.05; 0.08] mg/l for 400 mg x 1/day and 200 mg x 3/day, respectively. Median hydroxychloroquine ELF concentrations were of 3.74 [1.10; 7.26] mg/l and 1.81 [1.20; 7.25] for 400 mg x 1/day and 200 mg x 3/day, respectively. The median ratio of ELF/plasma concentrations was of 40.0 [7.3; 162.7] and 21.2 [18.4; 109.5] for 400 mg x 1/day and 200 mg x 3/day, respectively. Exposure in the ELF is likely to be underestimated due to the concentrations of plasma hydroxychloroquine. In clinical practice, low plasma concentrations should not induce an increase in drug dosage because the lung exposure may already be high.

KEYWORDS: COVID-19, hydroxychloroquine, plasma drug monitoring, bronchoalveolar lavage (BAL)

1. Introduction

Based on the *in vitro* work carried out against SARS-CoV-2 and preliminary clinical data, hydroxychloroquine is currently being used in the management of COVID-19 patients [1, 2]. Hydroxychloroquine may have an antiviral action through three main mechanisms: (1) viral entry prevention, (2) impairment of viral replication and (3) pleiotropic action on the human immune system through immuno-modulating activity [3]. The various *in vitro* studies have shown that the EC_{50} of hydroxychloroquine ranged from 0.72 to 4.4 µM (i.e. 0.241 to 1.4 mg/l) at 48 hours and 72 hours post-infection, respectively [3-5]. Pending the results of robust clinical trials and due to the lack of pharmacokinetic-pharmacodynamic information in COVID-19 patients, and in accordance with the National French Team, AC43-ANRS/STP-SFPT, different dosage regimens were applied in Toulouse University Hospital (France) (200 mg x 3 / day; 400 mg x 2 on day 1 then 200 mg x 3 / day; 400 mg x 2 on day 1 then 400 mg / day; and at least for ICU patients, 600 mg x 2 on day 1 then 400 mg/day) in order to reach pharmacokinetic equilibrium as quickly as possible [6].

On 2 April 2020, the French Ministry of Health imposed a dosage regimen identical to the one used in systemic lupus erythematosus (SLE; no loading dose and 200 mg x 3/day) for patients treated outside the context of a clinical trial.

Regardless of the dosage regimen, plasma concentration was monitored in order to evaluate individual drug exposure. It has been proven that the plasma concentrations measured in COVID-19 patients tended to be lower than the values reported in SLE patients, in particular for the standard regimen of “200 mg x 3/day” [7]. These preliminary results suggest that hydroxychloroquine concentrations are unlikely to be adequately predicted using the pharmacokinetic models derived from patients receiving hydroxychloroquine for SLE or rheumatoid arthritis treatment [8].

As the apparent volume of distribution of hydroxychloroquine is so large in volunteers as well as in malaria patients (≈ 5 000 l for the blood volume of distribution and ≈ 40 000 l for the plasma volume, at steady state) [9, 10], it can be suggested that the hydroxychloroquine gets trapped in the red cells and granulocytes [9, 11, 12] and probably in various tissues [10]. It could be assumed that the same occurs for COVID-19 patients. Consequently, it is natural to question the concentration of hydroxychloroquine at the infectious site (i.e. the lung) [13]. Unfortunately, this information is not available. A lung biopsy is the most informative approach. Even if a biopsy is a mixture of both intra- and extra-cellular matrices usually homogenised so as to determine a mean concentration [14], hydroxychloroquine gets trapped in the cells suggesting this drug is more likely present in the cells rather than outside. However, this option would be highly intrusive and an alternative approach would be to evaluate the hydroxychloroquine concentration in lung epithelial lining fluid (ELF) at the bedside of intensive care unit (ICU) patients [15, 16].

Drug concentration in ELF can be inferred based on the concentration of hydroxychloroquine in the bronchoalveolar lavage (BAL) fluid and the concentration of urea both in the plasma and the BAL fluid [17]. This method is not new and is usually applied to explore the lung diffusion of antibiotics in ICU patients. Consequently, when available, BAL fluid can be used as a kind of "quality control" used to obtain information on the degree of impregnation in the lung for a short period (10-15 days) of treatment.

The aim of our study was to evaluate hydroxychloroquine in the lung's ELF in COVID-19 patients in order to estimate the level of lung exposure.

2. Patients and Methods

This study is a retrospective, observational, multicentre, pharmacokinetic study of hydroxychloroquine in critically ill patients. We included all intubated COVID-19 patients treated with crushed hydroxychloroquine tablets, regardless of the dosage administered by nasogastric tube.

According to the guidelines established by the French National AC43-ANRS/STP-SFPT Team in March/April 2020, blood samples were collected at different time points (from 48h to 192h) during clinical management of COVID-19 patients after hydroxychloroquine initiation and 30 minutes before drug administration (i.e. trough concentration). In plasma, the steady state is supposed to be reached in 48 hours [18].

As part of our standard practice for monitoring patients with acute respiratory distress syndrome (ARDS) who are at high risk for infectious complications, and in this particular context of COVID-19, a mini-bronchoalveolar lavage (BAL) (twice 20 ml of physiological saline) was systematically performed 7 days (+/-2 days) after treatment initiation or in the event of a new respiratory degradation, for microbiological monitoring purposes (bacteriology, mycology and viral replication of SARS-CoV-2). A leftover volume of approximately 500 µl remained after these microbiological investigations, which was used to determine the hydroxychloroquine concentration. BAL sampling was carried out between two dose administrations, with no specific time imposed. Except for one case, only the BALs for which plasma determinations were performed within 48 hours before or after collection were included.

Hydroxychloroquine in plasma and BAL fluid concentrations were determined using a chromatographic analytical method, validated as per FDA guidelines. All BAL sample

preparations included a protein precipitation and a virus inactivation step in a methanol solution. The HCQ dosage method presents a lower limit of quantitation (LLOQ) of 0.05 mg/l in the plasma and 0.01 mg/l in the BAL fluid, an upper limit of quantitation (ULOQ) of 2 mg/l in the plasma and the BAL fluid, and an intra- and inter-day variability of <4% and <10% respectively. When plasma concentrations were <LOQ, the value was set to 0.025 mg/L (i.e. half the LLOQ). The plasma samples were stored at +4°C before analysis, for a period of 24h maximum. The BAL fluid samples were stored at -80°C before analysis, for a period of 30 days maximum. Previous studies have shown that hydroxychloroquine was stable in the whole blood under these conditions [19]. As whole blood is a complex matrix, in which xenobiotics tend to be less stable than other biological fluids, we considered that hydroxychloroquine was also stable in plasma and BAL fluid in these conditions.

Urea is used as an endogenous marker of ELF because urea, a small and relatively nonpolar molecule, can freely travel across membranes to reach the outer surfaces of alveoli. The concentration of urea in ELF ($Urea_{ELF}$) is considered to be same as in the serum urea ($Urea_{serum}$) concentration, implying complete distribution. Therefore, the volume of ELF (V_{ELF}) is adjusted for excess exogenous water using the following equation:

$$V_{ELF} / V_{BAL} = Urea_{BAL} / Urea_{plasma}$$

Knowing (1) the concentration of hydroxychloroquine measured in the BAL (HCQ_{BAL}), (2) the volume of BAL collected (V_{BAL}), the estimated ELF volume (V_{ELF}), it is then possible to determine the concentration of hydroxychloroquine in ELF (HCQ_{ELF}) using the following formula:

$$HCQ_{ELF} = V_{BAL}/V_{ELF} \times HCQ_{BAL} = (Urea_{plasma} / Urea_{BAL}) \times HCQ_{BAL}$$

Plasma urea levels were determined using an automated enzymatic method, validated as per FDA guidelines. This method presents a LLOQ of 0.5 mmol/l, an ULOQ of 15 mmol/l and an intra- and inter-day variability < 2% and <3%, respectively. Safety practices require a greater

level of caution when handling respiratory specimen from SARS-CoV-2 positive patients [20]. Thus, urea concentrations in BAL was assayed using a gas chromatography – a mass spectrometry method which included a protein precipitation and virus inactivation step in a methanol solution. A LLOQ of 0.1 mmol/l, and a ULOQ of 20 mmol/l were achieved. Precision assays showed an intra-day variability < 9% and an inter-day variability < 10%. BAL fluid samples were stored at -80°C before analysis, for a period of 60 days maximum. Previous studies have shown that urea was stable in serum in these conditions [21].

Continuous data was expressed as median (25th-75th percentiles) and categorical variables in numbers (percentages). The relationship between the plasma and ELF concentrations and the other parameters was assessed by simple linear regression. The analysis was performed using the MedCalc®15 statistical software program (Ostend, Belgium). $p < 0.05$ was considered to be statistically significant.

This study is entered in the Toulouse University Hospital register of retrospective studies (registration number: RnIPH 2020-33) and is covered by MR-004 (CNIL number: 2206723 v 0). This study was approved by Toulouse University Hospital and ethical requirements were entirely respected.

3. Results

3.1 Population

Twenty-eight hydroxychloroquine plasma and BAL fluid concentrations from 22 patients were measured (Table 1). The median patient age was of 60 [interquartile range (IQR) 53-70] years, and 91% of the patients were male. The median body mass index (BMI) was of 28 [IQR 26-31] kg/m^2. The median SAPS II and SOFA scores pertaining to the included patients were of 37 [IQR 32-46] and 6 [IQR 3-7] respectively, indicating a critically ill patient population.

3.2 Hydroxychloroquine trough concentrations

The values of the BAL fluid hydroxychloroquine concentration were determined 7 to 12 days after treatment initiation. For one point, the time from blood collection to BAL was 9.8 days, but the plasma concentration was at stead-state.

The median hydroxychloroquine plasma concentrations were of 0.09 [0.06; 0.14] mg/l and 0.07 [0.05; 0.08] mg/l for 400 mg x 1/day and 200 mg x 3/day, respectively. The median hydroxychloroquine ELF concentrations were of 3.74 [1.10; 7.26] mg/l and 1.81 [1.20; 7.25] mg/l for 400 mg x 1/day and 200 mg x 3/day, respectively. The median ratio of ELF/plasma concentrations were of 40.0 [7.3; 162.7] and 21.2 [18.4; 109.5] for 400 mg x 1/day and 200 mg x 3/day, respectively (Table 2).

The relationship between ELF and the plasma hydroxychloroquine concentration is presented in Figure 1.

No relationship was observed between the measured hydroxychloroquine concentrations and the biological parameters characterising renal and hepatic functions (Supplementary Table 1).

4. Discussion

As previously reported for many anti-infective drugs used to treat pulmonary infections, ELF concentration gives information on the intra- and extracellular lung exposure [15, 16, 22]. However, this approach is essentially reserved for clinical research as the therapeutic monitoring of anti-infective drugs in ELF is determined by practical and organisational constraints. Firstly, performing a BAL requires that the operator be trained and that the patient be stable enough to tolerate the serum injection in such way that it is only exceptionally carried out on non-intubated patients. Secondly, drug quantification has to be performed in an unconventional matrix (i.e. BAL) with a very sensitive analytical method (i.e. more often LC-MS/MS). In the special case of COVID-19 patients, the BAL is contaminated by SARS-CoV-2, thus imposing a specific and time consuming pre-analytical process.

We were able to gather all these conditions in order to assess whether all ELF concentrations are higher than plasma concentrations, despite the variability of ELF values. The significant variability in ELF concentrations may be explained in part by the BAL sampling. In fact, even if the injection volume were standardised (2 x 20 ml), the dwell time and the aspiration pressure cannot be strictly identical [23]. Cells can also be part of the ELF, especially macrophages, and may be lysed when measuring the drug concentration. Depending on their quantity, the lysis of these cells may induce an increase in the hydroxychloroquine concentration [11, 17]. As no measurement of the cell burden in the BAL sample was performed due to insufficient BAL volumes available for pharmacokinetic exploration, this lack of information has to be considered as a limitation of our study. Indeed, the cell burden in the BAL sample is likely associated with the ELF hydroxychloroquine concentration (i.e. the more the cells, the higher the concentrations).

Collecting blood and BAL samples at a different moment (day and/or time) and the potential post-dose discrepancy between the blood sample and the collection of BAL, could appear as limitating the interpretation of the ELF/plasma concentrations ratios. However, hydroxychloroquine presents a large volume of distribution with deep compartments (i.e. lung, spleen, melanin-containing tissues… [3] leading to different kinetic profiles in the plasma and lung tissue [24]. Indeed, half-life elimination is likely to be short in the plasma of COVID-19 patients [7, 18] as opposed to deep compartments. The blood samples have always been taken at steady state while the BALs could be collected always after the plasma concentration peak. Indeed, the staff in charge of carrying out the BALs was warned of this constraint. Furthermore, a flat kinetic profile was expected in the lung tissue [14]. As a consequence, it seems reasonable to suppose that ELF/plasma concentrations ratios do not change between administrations, once the steady state has been reached. In fact, the ideal solution would consist in determining the area under the time-concentration curve (AUC) in both plasma and BAL matrices at steady state and to calculate the AUC ratio. This option is not feasible and not ethical for critically ill patients presenting ARDS because multiple BALs would alter the gas exchanges between alveoli and capillaries. It would worsen PaO2/FiO2 ratios (the ratio of arterial oxygen partial pressure (PaO2 in mmHg) to fractional inspired oxygen (FiO2 expressed as a fraction)).

However, as our data was retrospectively collected from a small population of ICU patients, one limitation of our study is that the inter-individual variability of the plasma and ELF hydroxychloroquine concentrations likely under/overestimates the actual inter-individual value.

Our results show that hydroxychloroquine concentrations in the lung are higher than in the ELF. Passage from the blood compartment to the ELF involves passing through the pulmonary epithelial cells (i.e. prime target for the replication of SARS-CoV-2 [17, 25, 26])

241 in which hydroxychloroquine is most likely accumulated with pharmacokinetic hysteresis.
242 Hydroxychloroquine's mechanism of action is poorly elucidated, but includes, among others,
243 the increase in endolysosomal pH necessary for viral fusion. The initial fusion between the
244 viral and the cellular membranes (e.g. lung epithelial cells) requires an interaction between the
245 surface proteins of the two partners, and this interaction can only take place under particular
246 acidic conditions, through the phenomenon of endocytosis. The inability to obtain the ideal
247 pH can block this process, and it is probably through this means that hydroxychloroquine may
248 act. Other properties may be involved: modification in the glycosylation of angiotensin
249 converting enzyme-2, the receptor that SARS-CoV-2 uses to enter the cells and/or post-
250 translational modification of some viral proteins [27]. In addition, viral invasion may also
251 trigger a massive margination of the phagocytic cells to the infection site, which may deliver
252 increased amounts of hydroxychloroquine [28]. But, in the absence of clear information on
253 the influence of the inflammatory status reported in COVID-19 patients as to the
254 accumulation of hydroxychloroquine in lungs, this point should be considered as a limit of
255 our study.
256
257 Plasma concentration is not predictive of lung concentration, as shown in Figure 1. Therefore,
258 drug dosage determinations in studies assuming equilibrium between epithelium and plasma
259 concentrations may lead to overly high dosages [29]. Furthermore, in clinical practice, low
260 plasma concentrations should not induce an increase in the drug dosage because the lung
261 exposure may already be high. The various *in vitro* studies have shown that the EC_{50} of
262 hydroxychloroquine ranged from 0.72 to 4.4 µM (i.e. 0.241 to 1.4 mg/l) at 48 hours and 72
263 hours post-infection, respectively [3-5]. However, EC_{50} could be determined in Vero cell
264 lines, but not in a human epithelial cell model [4]. This discrepancy may explain that
265 hydroxychloroquine has not shown efficacy in clinical trials [29]. Thus, whether for a dose of

400 mg x 1 /day or 200 mg x 3 /day, the median ELF concentration of hydroxychloroquine is above the maximum EC_{50} value. Therefore, both regimens lead to a median lung exposure that could be sufficient to eradicate the virus. However, the heterogeneity of EC_{50}s raises the problem of selecting the "right" threshold used to determine the dosage, in particular through modelling techniques. In conclusion, despite all its imperfections, BAL fluid provides a rough idea of lung exposure.

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Declarations

Funding: This study was supported by internal funding.

Competing interests: None to declare

Ethical Approval: This study was entered in the Toulouse University Hospital register of retrospective studies (registration number: RnIPH 2020-33) and is covered by MR-004 (CNIL number: 2206723 v 0). This study was approved by Toulouse University Hospital and all ethical requirements were complied with.

Authors' contributions: SR, DC, JMC and PG conceptualised the research aims, designed the study, and took responsibility for the integrity of the data and the accuracy of the data analysis. PG, HV, DR, VM and BG contributed to the acquisition of data. TL, SB, ML and CM performed HCQ concentration determinations in BALs and plasma. DC and JMC

performed the statistical analysis. SR, DC, JMC and PG wrote the first draft of the paper, and other authors provided comments and approved the final manuscript.

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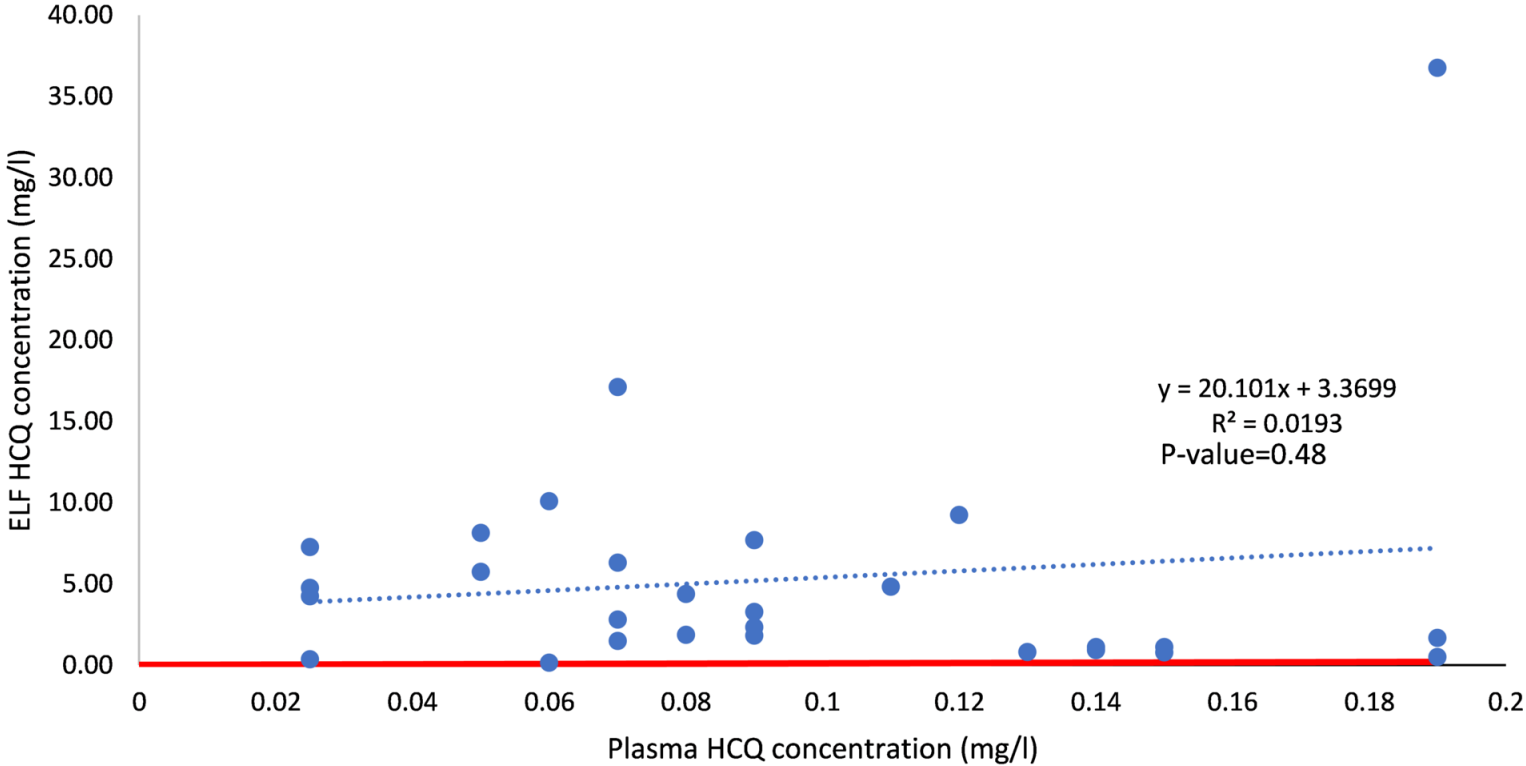
Table legends

Table 1: Socio-demographic and clinical data of 22 severe COVID-19 patients treated with hydroxychloroquine and for whom a bronchoalveolar lavage was performed (BMI: body mass index; CKD-EPI: Chronic Kidney Disease EPIdemiology; IQ: interquartile; SAPS II: simplified acute physiology score; SOFA: Sepsis-related Organ Failure Assessment).

Table 2: Hydroxychloroquine (HCQ) plasma and epithelial lining fluid (ELF) concentrations (IQR: interquartile range).

Figure legend

Figure 1: Relationship between lung epithelial lining fluid (ELF) and plasma hydroxychloroquine (HCQ) concentrations. The red line is the identity line (i.e. the plasma and ELF concentrations are equal). The dots above the red line indicate that the concentrations in the ELF are higher than the plasma concentrations (ELF/plasma HCQ concentrations: 38.072 [8.338 to 138.521]). This figure shows that (i) even with small plasma concentrations, the ELF concentration can be high and (ii) the plasma concentration is a poor predictor of the ELF concentration.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0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
Plasma HCQ concentration (mg/l)
y = 20.101x + 3.3699
R² = 0.0193
P-value=0.48

Table 1: Socio-demographic and clinical data of 22 severe COVID-19 patients treated with hydroxychloroquine and for whom a bronchoalveolar lavage was performed (ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: body mass index; CKD-EPI: Chronic Kidney Disease EPIdemiology; D0: day 0 of the hydroxychloroquine initiation, D7: day 7 after hydroxychloroquine initiation; IQR: interquartile range; SAPS II: simplified acute physiology score; SOFA: Sepsis-related Organ Failure Assessment).

All Patients (n=22)	**Median [IQR]**	**Min - Max**
Age	59.5 [53 to 70]	30 - 81
BMI	28.3 [26 to 31.3]	20.7 - 37
SAPS II	37 [32 to 46]	8 - 76
SOFA score	6 [3 to 7]	2 - 14
Protidemia (g/L) D7	61 [59 to 68]	50 - 77
AST(UI/L) D7	65[69 to 179]	28 - 135
ALT (UI/L) D7	99 [69 to 179]	18 - 257
Bilirubin (µmol/L) D0	7.6 [5.15 to 11.2]	4 - 29
CKD-EPI D7 (mL/min/1.73 m²)	97 [60.5 to 105.8]	9 - 123
Duration of invasive ventilation (day)	19.5 [11 to 28]	0 - 22

Table 2: Hydroxychloroquine (HCQ) plasma and epithelial lining fluid (ELF) concentrations (IQR: interquartile range).

	All dosages		400mg x1/day		200 mgx3/day	
	Median [IQR]	**Min - Max**	**Median [IQR]**	**Min - Max**	**Median [IQR]**	**Min - Max**
Plasma HCQ concentrations (mg/L)	0.09 [0.06 to 0.14]	0.03 – 0.19	0.09 [0.06 to 0.14]	0.03 – 0.19	0.07 [0.05 to 0.08]	0.03-0.09
ELF HCQ concentrations (mg/L)	3.03 [1.10 to 6.78]	0.13 – 36.75	3.74 [1.1 to 7.26]	0.13 – 36.75	1.807 [1.2 to 7.25]	0.34 – 10.08
ELF/plasma HCQ concentrations	38.07 [8.34 to 138.52]	2.1 - 290.4	39.96 [7.33 to 162.66]	2.1 - 290.4	21.22 [18.41 to 109.49]	13.4 - 168