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Chloroquine for treatment of COVID-19 results in subtherapeutic exposure and prolonged QTc intervals

Editor: Dr Jim Gray

At the end of 2019 in Wuhan, China, the SARS-CoV-2 virus emerged as a cause of severe respiratory illness. Not long after, the World Health Organization declared COVID-19 a pandemic. The impact of COVID-19 has resulted in a global race to find treatments. Preclinical studies indicated antiviral activity of the antimalarial drug chloroquine against the novel coronavirus. One study showed 90% inhibition (IC_{90}) of viral replication at a concentration of 6.9 μM [1]. Another study showed a higher IC_{90} of chloroquine (>10 μM) [2]. The results of these preclinical studies led to the wide scale repurposing of chloroquine as a treatment for COVID-19. It remains unknown whether the best-case therapeutic threshold of 6.9 μM determined in preclinical studies is achieved in COVID-19 patients and whether this threshold can be directly translated to the clinic. There is increasing information disputing the clinical benefit of chloroquine in COVID-19.

We investigated the pharmacokinetics and cardiac safety of chloroquine in hospitalized patients with COVID-19 who received this treatment in five hospitals in The Netherlands. The medical ethical review board CMO region Arnhem Nijmegen (Nijmegen, The Netherlands) and the COVID-19 research committee at Leiden University Medical Center (The Netherlands) decided that our observational study was not subject to The Dutch Medical Research Involving Human Subjects Act (WMO). Chloroquine was administered at a loading dose of 600 mg followed by 300 mg 12 hours later on day 1, then 300 mg twice per day for a total treatment duration of 5 days, regardless of body weight [3]. Electrocardiography with Bazett QT interval correction was performed at baseline and during treatment at the discretion of the treating physician. Plasma concentrations of chloroquine and its metabolite, desethylchloroquine were measured in samples collected as a 4-point pharmacokinetic curve over an 8-hour interval, or in randomly collected surplus samples from routine laboratory monitoring. Plasma concentrations of chloroquine and desethylchloroquine were measured using a validated bioanalytical assay [submitted for publication]. In addition, chloroquine and metabolite protein-unbound concentrations were determined in 20 random samples. Lastly, the unbound fractions of chloroquine and desethylchloroquine were determined at a concentration of 1 and 10 μM in six-fold in the same cell medium as that used by Wang et al. [1].

A total of 83 patients were included, with a median (interquartile range [IQR]) age of 65 (57-73) years. All but two of the patients were admitted to the intensive care unit, 60% of the patients were male, and the median (IQR) body mass index (BMI) was 26.8 (24.9-30.5). The measured concentrations of chloroquine, desethylchloroquine, and the sum of both chloroquine and its metabolite are depicted in Figure 1. None of the measured concentrations of chloroquine, nor the sum concentration of chloroquine and its metabolite reached the best-case antiviral in vitro IC_{90} on any treatment day. Furthermore, the median (IQR) protein-unbound fractions of chloroquine and desethylchloroquine in clinical samples were 18.3 (16.2-22.4)% and 20.2 (18.2-25.2)%, respectively. The median (IQR) unbound fractions of chloroquine and desethylchloroquine in cell medium with 10% fetal bovine serum at the 1 μM concentration were 62.0 (58.4-633)% and 71 (IQR 69.1-73.0)%, respectively. At 10 μM these were 84.1 (83.8-84.4)% and 88.3 (87.6-88.7)%, respectively. QTc measurements at baseline and during chloroquine treatment were available in 41 patients. In 14 of these patients a ΔQTc >60 ms was observed after initiation of chloroquine. QTc measurements during chloroquine treatment were available in 69 patients and a QTc $\geq$500 ms during treatment was observed in 32 (46%) of these patients. Torsade de pointes was not reported. No statistically significant differences in plasma concentrations of chloroquine were observed in patients with or without QTc >500 ms.

These results show that treatment with chloroquine does not lead to plasma concentrations reaching the best-case IC_{90} of 6.9 μM. Chloroquine is metabolized in vivo to desethylchloroquine [4], which is a cationic amphiphilic molecule that accumulates in lysosomes and has broad antiviral activity and therefore is likely to have antiviral properties against SARS-CoV-2 [5]. In this study, even the sum of the individual chloroquine and desethylchloroquine concentrations did not exceed the 6.9 μM target (Figure 1). Furthermore, chloroquine is known to bind to plasma proteins. In the study population, the unbound fraction of chloroquine and desethylchloroquine in plasma was approximately 20%, whereas the unbound fraction in cell medium was much higher at 60-80%. Consequently, the gap between the pharmacologically active concentrations achieved in vivo and the in vitro IC_{90} is even larger than presented in Figure 1 because of this discrepancy. Chloroquine accumulates in lung tissue [6], which is lysosome rich, resulting in a relatively high overall abundance of chloroquine in the lung [7]. However, as chloroquine accumulation into the lysosomes of the lung is likely to be similar to that in other tissues due to the physicochemical properties of chloroquine, it is unlikely that effective exposure is reached in lung tissue. Although a simple solution to subtherapy would be a higher dose of chloroquine, QTc prolongation is a serious concern. High-dose chloroquine has recently been shown to be associated with increased mortality and life-threatening QTc prolongations compared with low-dose chloroquine in patients with COVID-19 [8]. Our findings cast serious doubt on the role of chloroquine for treatment of COVID-19 and underline the need for dose-finding studies before rushing a drug from the bench to the bedside of a vulnerable population.

Tables 1 and 2

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Table 1
Patient Demographics

Variable	No (%) of patients (N=83)
Demographics	
Age, y	
Median (IQR)	65 (57-73)
>65	41 (49)
Sex	
Male	60 (72)
Female	23 (28)
Weight, kg	
Median (IQR)	82 (75-95)
BMI	
Median (IQR)	26.8 (24.9-30.5)
Reported comorbidities*	
Serious heart condition	15 (18)
Chronic lung disease or	6 (7)
moderate to severe asthma	9 (11)
Immunocompromized	0 (0)
Obese	
BMI >30	23 (29)
BMI >40	4 (5)
Diabetes	12 (14)
Chronic kidney disease	2 (2)
Liver disease	0 (0)
Chloroquine treatment	
Days of therapy	
1	3 (4)
2	2 (2)
3	6 (7)
4	12 (14)
5	48 (58)
6	9 (11)
7	2 (2)
8	1 (1)
Admitted to	
General ward	2 (2)
Intensive care unit	81 (98)
Maximum number of simultaneous prescriptions of QT-prolonging comedication during chloroquine treatment	
0	23 (28)
1	47 (55)
2	10 (12)
3	3 (4)
Drug	
Propofol	48 (59)
Ciprofloxacin	14 (17)
Erythromycin	13 (16)
Amiodarone	2 (2)
Haloperidol	2 (2)
Sotalol	1 (1)
Renal function (CKD-EPI, mL/min)	
Median (IQR)	73 (52-90)
Liver	
ASAT >2x ULN	32 (42% of 75)
ALAT >2x ULN	10 (13% of 75)
Outcome	
Deceased	19 (23)

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; IQR, interquartile range; ULN, upper limit of normal

* Comorbidities reported at admission, based on CDC guidance; Groups at higher risk for severe illness

Table 2
QTc time

Parameter	No (%) of patients (N=83)
Number of patients with ECG data available	79 (95)
Number of patients with ECG measured at baseline	51 (61)
QTc, Median (IQR)	450 (425-482)
>500 ms	7 (14% of 51)
Number of patients with ECG measured during treatment	69 (83)
QTc, Median (IQR)	476 (445-501)
QTc >500 ms at any time during treatment	32 (46% of 69)
QTc >600 ms at any time during treatment	7 (10% of 69)
Number of patients with ECG measured at baseline and during treatment	41 (49%)
Maximum QTc prolongation compared with baseline (ms), Median (IQR)	41 (6-88)
QTc prolongation >60 ms compared with baseline	14 (34% of 41)

ECG, electrocardiogram; IQR, interquartile range

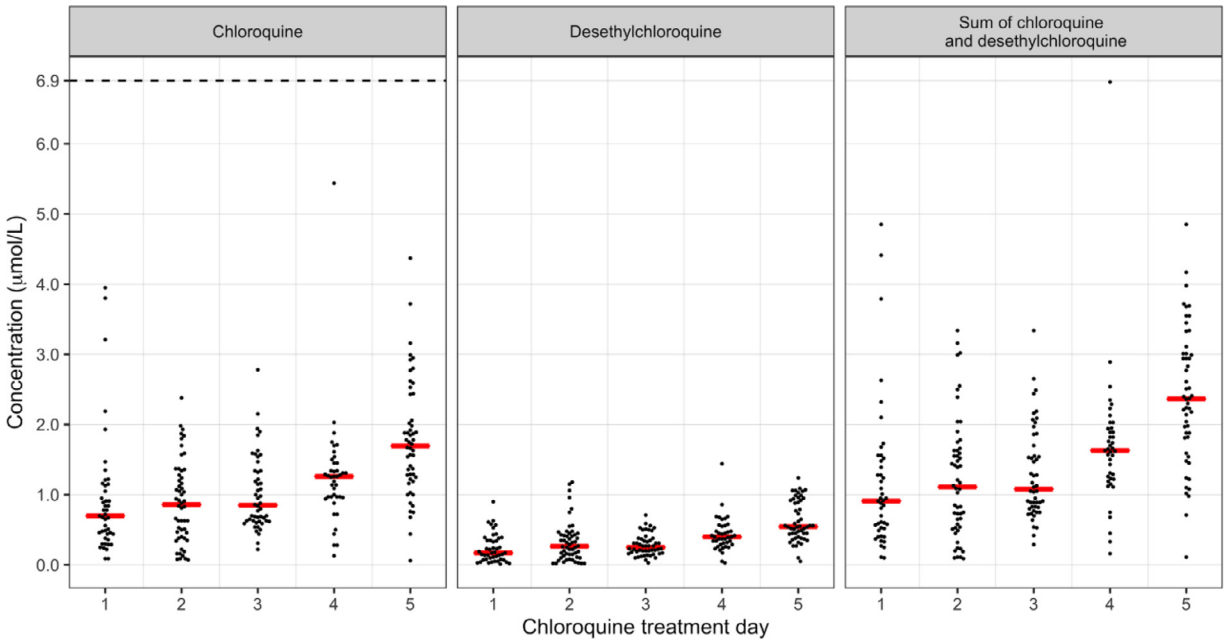

Figure 1. Left panel: Total (free and protein-bound) concentration of chloroquine in μmol/L for every treatment day of chloroquine. Middle panel: metabolite desethylchloroquine in μmol/L. Right panel: sum of chloroquine and desethylchloroquine in μmol/L. Dashed line in left panel represents the IC_{90} from the study by Wang et al [1]. All concentrations are shown irrespective of time of day.

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