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Effect of hydroxychloroquine on the cardiac ventricular repolarization: A randomized clinical trial

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Aims: Hydroxychloroquine has been suggested as possible treatment for severe acute respiratory syndrome-coronavirus-2. Studies reported an increased risk of QTcF-prolongation after treatment with hydroxychloroquine. The aim of this study was to analyse the concentration-dependent effects of hydroxychloroquine on the ventricular repolarization, including QTcF-duration and T-wave morphology.

Methods: Twenty young (≤ 30 y) and 20 elderly (65–75 y) healthy male subjects were included. Subjects were randomized to receive either a total dose of 2400 mg hydroxychloroquine over 5 days, or placebo (ratio 1:1). Follow-up duration was 28 days. Electrocardiograms (ECGs) were recorded as triplicate at baseline and 4 postdose single recordings, followed by hydroxychloroquine concentration measurements. ECG intervals (RR, QRS, PR, QTcF, J-Tpc, Tp-Te) and T-wave morphology, measured with the morphology combination score, were analysed with a prespecified linear mixed effects concentration–effect model.

Results: There were no significant associations between hydroxychloroquine concentrations and ECG characteristics, including RR-, QRS- and QTcF-interval ($P = .09$, $.34$, $.25$). Mean $\Delta\Delta$ QTcF-interval prolongation did not exceed 5 ms and the upper limit of the 90% confidence interval did not exceed 10 ms at the highest measured concentrations (200 ng/mL). There were no associations between hydroxychloroquine concentration and the T-wave morphology ($P = .34$ for morphology combination score). There was no significant effect of age group on ECG characteristics.

Conclusion: In this study, hydroxychloroquine did not affect ventricular repolarization, including the QTcF-interval and T-wave morphology, at plasma concentrations up to 200 ng/mL. Based on this analysis, hydroxychloroquine does not appear to increase the risk of QTcF-induced arrhythmias.

KEYWORDS

cardiac ventricular repolarization, concentration–effect analysis, electrocardiogram, hydroxychloroquine, QT-interval

All authors confirm that the Principal Investigator for this paper is M. Moerland and that he had direct clinical responsibility for subjects.

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1 | INTRODUCTION

Since January 2020, a pandemic of the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) has been expanding. Worldwide, over 4 million deaths associated with SARS-CoV-2 infection have been documented.¹ Being a new viral infection, no treatment was available initially, but **hydroxychloroquine** was suggested to potentially have a therapeutic or prophylactic effect on SARS-CoV-2 infection after promising results showing inhibition of virus replication in *in vitro* studies.^{2,3} Therefore, many SARS-CoV-2 infected patients were treated with hydroxychloroquine without proven clinical benefit of this drug, particularly during the first months of the pandemic.⁴ After inconclusive results of initial clinical studies on the therapeutic and prophylactic efficacy of hydroxychloroquine for SARS-CoV-2, the World Health Organization eventually advised against its use for this infection as large randomized controlled trials showed that there was no clinical benefit of hydroxychloroquine for SARS-CoV-2 patients.⁵⁻¹⁰

Hydroxychloroquine was originally developed as antimalarial drug during World War II and has been used both as antimalarial drug and in the treatment of connective tissue diseases for decades, with only very few reports of cardiac death after use of hydroxychloroquine.¹¹⁻¹⁴ Nonetheless, reports of QT-prolongation, torsade de pointes (TdP) and even cardiac death after use of hydroxychloroquine against SARS-CoV-2 infection have raised concerns about its proarrhythmic effects.^{5,15-19} More specifically, in a meta-analysis including 28 observational studies on the effect of hydroxychloroquine in SARS-CoV-2 patients, the frequency of TdP was 0.06% and the frequency of arrhythmogenic death was 0.69%, although it should be considered that hydroxychloroquine was combined with other QT-prolonging drugs in the majority of the studies (20 out of 28).²⁰ Recent observations on the cardiac side effects of hydroxychloroquine are in contrast to other studies that reported an association between use of hydroxychloroquine and a reduced cardiovascular risk.^{21,22} However, these studies, reporting a reduced cardiovascular risk associated with hydroxychloroquine use, were executed in patients with rheumatic diseases, who use hydroxychloroquine chronically,^{21,22} making the results difficult to generalize to the population of SARS-CoV-2 patients, who use hydroxychloroquine for a short period. The renewed attention for this drug in regard to the SARS-CoV-2 pandemic calls for a comprehensive characterization of the effects of hydroxychloroquine on the electrocardiogram (ECG), in line with current requirements for obtaining a waiver for conducting a thorough QT study for new drugs (E14, R3 update),²³ relating the pharmacokinetic concentration to the QTc-effect. As described by Darpo *et al.* and Cavero *et al.*, exposure-response analysis of small studies (20 subjects) within healthy subjects is sufficient to detect QT-effects, although it is typically required to evaluate QT-effects in supratherapeutic dose levels.^{24,25} More specifically, Gaitonde *et al.* showed that results of phase I studies (including minimal 28 subjects) can be used to obtain results consistent with results of thorough QT studies.²⁶ Information of this concentration-response (i.e. concentration-QTc effect)

What is already known about this subject

- Hydroxychloroquine has been used as anti-malarial and anti-rheumatic drug for decades, with only very few reports of cardiac death after use of this drug. The current severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic has raised renewed attention on hydroxychloroquine.
- Concerns were raised on the proarrhythmic effects of hydroxychloroquine, that are presumably caused by blockage of the cardiac hERG channel.
- This study analysed the effect of hydroxychloroquine, dosed in a regular regimen for SARS-CoV-2 patients, on the ventricular repolarization in a concentration-effect analysis.

What this study adds

- There were no significant associations between hydroxychloroquine concentration and electrocardiogram characteristics, including QTcF-interval and T-wave morphology, measured with a morphology combination score. Based on this analysis, hydroxychloroquine does not significantly affect the QTcF-interval and does not significantly increase the risk of arrhythmias in the studied dosing regimen and population.
- This outcome can be explained by the maximal plasma concentration of this dosing regimen, that is not high enough to exert hERG channel inhibition.
- We hypothesize that the observed QTcF-interval prolongation in SARS-Cov-2 patients using hydroxychloroquine is attributable to their general health status and comedication.

analysis is not only of importance regarding the SARS-CoV-2 pandemic, as hydroxychloroquine is used in the standard treatment of a broad range of diseases.

At time of development of hydroxychloroquine, proarrhythmic properties of new drugs were not as extensively studied as nowadays.²⁷ Although proarrhythmic properties of drugs are evaluated by measuring its QT-prolonging characteristics, the QT-interval is not an ideal marker for proarrhythmic risks as the extent of QT-prolongation is poorly correlated with TdP risk, and drugs that have a comparable rate of QT-prolongation can have different TdP incidences.^{28,29} Recently, a novel algorithm was developed to evaluate **hERG** effects on the ECG.²⁹ hERG channel block, associated with an increased TdP risk, has been shown to induce notches, asymmetry and flatness of T-waves.²⁹ These abnormalities can be summarized in the morphology

combination score (MCS), resulting in a measure of TdP risk independent of heart rate (Figure S1).^{28–32}

The present analysis aimed to investigate the concentration–effect association between hydroxychloroquine and ventricular repolarization, including QTcF-interval and T-wave morphology, and other electrocardiographic parameters, using a standardized linear mixed effects modelling approach.

2 | METHODS

We conducted a single-blind, randomized, placebo-controlled multiple dose study in 40 healthy male volunteers, comprising 20 young (18–30 y) and 20 elderly (65–75 y) male subjects. The study was conducted at the Centre for Human Drug Research in Leiden, The Netherlands, between June and September 2020, during the SARS-CoV-2 pandemic. All subjects gave written informed consent according to Declaration of Helsinki recommendations, prior to any study-related activity. The study was approved by the Independent Ethics Committee of the Foundation *Evaluation of Ethics in Biomedical Research* (Stichting Beoordeling Ethiek Biomedisch Onderzoek), Assen, The Netherlands and was registered in the Toetsingonline Registry under study number NL73816.056.20, and in the *trialregister.nl* registry under number NL8726.

2.1 | Subject selection

All subjects underwent medical screening, including medical history, physical examination, vital signs measurements, 12-lead ECG, urine analysis, drug screen and safety chemistry, coagulation and haematology blood sampling. Key inclusion criteria were: no clinically significant abnormalities during the investigations performed during screening and body mass index between 18 and 32 kg/m², key exclusion criteria were: a known hypersensitivity reaction to chloroquine, hydroxychloroquine or 4-aminoquinolines, abnormalities in the resting ECG (including QTcF-interval >450 ms), evidence of any active or chronic disease or condition and a positive SARS-CoV-2 test. Use of concomitant medication was not permitted during the study, or 14 days (or 5 half-lives) prior to the study drug administration, with exception of paracetamol.

2.2 | Study design

Subjects were randomized to receive either orally dosed hydroxychloroquine sulphate (plaquenil) or placebo, with a 1:1 ratio, in a single-blind fashion. Randomization codes were generated by a study-independent statistician and were placed in sealed emergency decoding envelopes. Tablets were dispensed by the pharmacy, according to the randomization list, in identical packaging. Study staff involved in dosing was not involved in performing PD-assays or

analysis of the data. Subjects received doses of 400 mg hydroxychloroquine or placebo at t = 0, 12, 24, 48, 72 and 96 hours, for a total dose of 2400 mg. This dose was the standard dosing regimen for moderate to severe SARS-CoV-2 patients in the Netherlands when the study was conceived (regular dosing regimens consisted of a total dose between 2000 and 3800 mg).³³ ECG recordings were performed at baseline (triplicate ECG), 3 and 27 hours and 5 and 10 days after the first hydroxychloroquine administration (total of 7 ECGs). Corresponding plasma hydroxychloroquine concentrations were measured resulting in a total of 4 PK measurements with matching ECGs postdose. All subjects had eaten a meal before having taken hydroxychloroquine, to minimize gastrointestinal adverse effects, which have been reported after hydroxychloroquine use.³⁴

2.3 | ECG analysis

The 10-second 12-lead ECGs were recorded with the subject in supine position after a 5-minute resting period and prior to the PK sampling. The 12-lead ECGs were recorded using an electrocardiograph (Marquette 2000/5500; General Electric Healthcare, Milwaukee, USA) and 10 disposable electrodes placed in the standard anatomical position. The ECG data were then processed by the Department of Health Science and Technology, Faculty of Medicine, University of Aalborg (Denmark) using the commercially available GE Healthcare Marquette 12SL ECG analysis program and the US Food and Drug Administration 510(k)-cleared GE research package QT Guard Plus (GE Healthcare, Wawatosa, WI, USA), which uses validated algorithms for measurement.³⁵ The software uses all simultaneous 12 leads to construct a representative median beat from non-ectopic PQRST complexes and measures intervals from the earliest onset in any lead to the latest offset in any lead. Parameters that were automatically assessed in the ECG were: RR, QT, PR, QRS, J–Tpeak (J–Tp), Tpeak–Tend (Tp–Te) intervals and additionally the MCS measure of T-wave morphology.³⁶ The QT interval was corrected for heart rate using the Fridericia method ($QTcF = QT / \sqrt[3]{RR}$). The J–Tp interval was corrected for heart rate using the formula ($JTpc = \frac{J-T_{peak}}{RR^{0.36}}$).

2.4 | Pharmacokinetic analysis

Blood sampling occurred after ECG sampling and the actual time-point of the drawn blood sample was recorded for the pharmacokinetic analysis. Hydroxychloroquine plasma concentrations were measured by Ardena Bioanalytical Laboratory (Assen, the Netherlands) using a validated liquid chromatography–tandem mass spectrometry method. The lower limit of quantification of the analysis was 5 ng/mL.

2.5 | Statistical analysis

Statistical analysis was performed using NONMEM Version 7.3 (ICON Development Solutions, Hanover, MD, USA) and R for Windows

version 3.6.1. Hydroxychloroquine concentrations and ECG measurements were matched by nominal time and the actual time deviation between both measurements was checked for outliers. Concentration vs. ECG parameters analysis was performed using the following prespecified model³⁷:

$$\Delta PD_{ijk} = \theta_0 + \theta_1 TRT + \theta_2 (PD_{i,0} - \overline{PD}_0) + \theta_3 NTIM_j + \theta_4 C_{ijk} * (1 + \theta_5 * Age\ group)$$

where ΔPD_{ijk} is the change from baseline (Δ) of either RR, PR, QRS, QTcF, J-Tpc, Tp-Te or MCS for subject i in treatment j at time k ; θ_0 is the population intercept; TRT is 1 for active and 0 for placebo; $PD_{i,0}$ is the individual baseline value calculated as the mean of the 3 predose measurements; \overline{PD}_0 is the overall mean of all baseline values; NTIM is the nominal (protocol) time as a factor; θ_4 is the slope for the assumed linear relationship between hydroxychloroquine concentration (C) in plasma and ΔPD ; and θ_5 is the effect of the age group (0 or 1) on the sensitivity to hydroxychloroquine. Subject was included as additive random effect on intercept and slope with a full omega block. If no successful covariance step was obtained, the random effects structure was reduced until the covariance step was successful. A significant concentration-effect relationship of hydroxychloroquine on any of the ECG parameters was judged by the level of significance of the slope parameter.

The adequacy of the prespecified direct effect linear model was evaluated prior to analysis using hysteresis plots and scatter plots of placebo-corrected ΔPD (obtained by subtracting the mean of the placebo group at each protocol time from the mean ΔPD) vs. hydroxychloroquine concentration overlaid with loess smooth line and linear regression line; and after analysis using goodness-of-fit plots and confidence interval (CI) visual predictive checks.

The final concentration-effect models were used to compute the placebo- and baseline-corrected ($\Delta\Delta$) $\Delta\Delta RR$, $\Delta\Delta PR$, $\Delta\Delta QRS$,

$\Delta\Delta QTcF$, $\Delta\Delta J-Tpc$, $\Delta\Delta Tp-Te$ and $\Delta\Delta MCS$ at a range of concentrations up to 200 ng/mL and at the geometric mean maximum plasma concentration.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, and are permanently archived in the Concise Guide to Pharmacology 2019/20: ion channels.⁵⁵

3 | RESULTS

Baseline characteristics can be observed in Table 1. Forty male subjects completed the study without dropouts. Mean \pm standard deviation age in the young group was 23 ± 2.1 years, and 68 ± 1.6 years in the elderly group. There were no relevant differences in baseline characteristics between the group of young and elderly subjects besides age. A total of 158 matched concentration-ECG observations were available for analysis, of which 79 from the active treated group. Hydroxychloroquine plasma concentrations were above the lower limit of quantification as shown in Figure 1 for all measurements. No apparent difference in systemic hydroxychloroquine exposure between age groups was identified.

3.1 | Pharmacodynamic effects

The exploratory analysis of all baseline-corrected ECG parameters showed a linear trend (Figures 2 and S2) and no evidence for non-linearity, saturable effects, or hysteresis was identified, allowing for the use of the prespecified model. An adequate fit to the data was

TABLE 1 Baseline characteristics of all subjects, and stratified by young and elderly subjects. Continuous variables are expressed as mean \pm standard deviation. Categorical variables are displayed as number (percentage)

	Overall <i>n</i> = 40	Young <i>n</i> = 20	Elderly <i>n</i> = 20
Age (y)	45.9 \pm 22.4	23 \pm 2.1	67.7 \pm 1.6
Male, <i>n</i> (%)	40 (100)	20 (100)	20 (100)
Height (cm)	182 \pm 7	183 \pm 7	181 \pm 6
Weight (kg)	79.3 \pm 8.6	76.8 \pm 8.6	81.6 \pm 7.8
Body mass index (kg/m ²)	24.0 \pm 2.5	22.9 \pm 2.0	25 \pm 2.5
Systolic blood pressure (mmHg)	126.9 \pm 10.5	122.3 \pm 8.0	131.4 \pm 10.7
Diastolic blood pressure (mmHg)	77.3 \pm 9.1	70.6 \pm 5.2	83.8 \pm 7.1
Baseline QTcF (ms)	415 \pm 11.6	408 \pm 11.6	421 \pm 7.9
Ethnicity: Caucasian, <i>n</i> (%)	40 (100)	20 (100)	20 (100)
Sodium (mmol/L)	141.1 \pm 1.4	141.4 \pm 1.1	140.7 \pm 1.6
Potassium (mmol/L)	4.4 \pm 0.2	4.4 \pm 0.2	4.4 \pm 0.2
Glucose (mmol/L)	5.0 \pm 0.4	4.8 \pm 0.4	5.2 \pm 0.3

obtained for all parameters in which the median and prediction interval of the model correctly described the distribution of the data. No structural misspecifications were identified in any of the models (Figure S3).

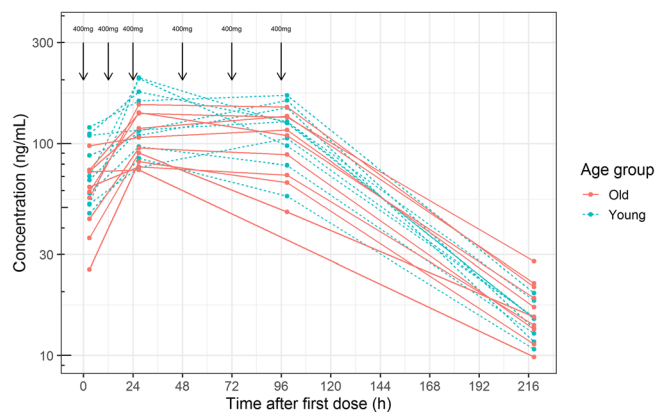


FIGURE 1 Plasma hydroxychloroquine concentrations for both age groups. Arrows indicate the time of dosing

The slope and the significance level of the linear concentration–effect relationship of hydroxychloroquine on different ECG-characteristics are shown in Table 2. The estimated slopes were scattered around 0 and there were no significant associations between the hydroxychloroquine concentration and any of the ECG parameters, including PR-, QRS- and QTcF-interval duration (slope estimate $\Delta\Delta\text{PR} = 0.010$, 95% CI -0.030 to 0.050 , $P = .32$; slope estimate $\Delta\Delta\text{QRS} = 0.005$, 95%CI -0.017 to 0.026 , $P = .34$; slope estimate $\Delta\Delta\text{QTcF} = 0.017$, 95%CI -0.033 to 0.068 , $P = .25$). As can be observed in both Table 2 and Figure 3, there were no significant associations between hydroxychloroquine concentration and RR-interval, QTcF-interval and the MCS (slope estimate $\Delta\Delta\text{RR} = -0.341$, 95%CI -0.841 to 0.160 , $P = .091$; slope estimate $\Delta\Delta\text{MCS} = 6.30 \times 10^{-5}$, 95%CI -2×10^{-4} to 4×10^{-4} , $P = .34$). For the other investigated parameters, reference is made to Figure S4. Notably, in the concentration range up to 200 ng/mL that was investigated, the mean QTcF interval prolongation did not exceed 5 ms and the upper limit of the 90% CI did not exceed 10 ms. There was also no association between hydroxychloroquine concentration and T-wave morphology indices. Asymmetry and

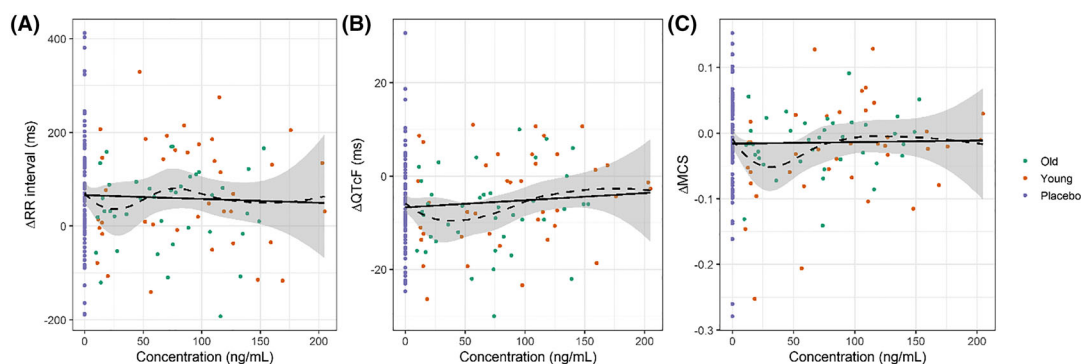


FIGURE 2 Scatter plots of hydroxychloroquine plasma concentration with corresponding ΔRR interval in ms, ΔQTcF interval in ms and ΔMCS . Solid black lines show linear regression and dashed lines show loess smooth of the data with corresponding 90% confidence interval in grey. Young subjects receiving hydroxychloroquine are represented by red dots; elderly subjects receiving hydroxychloroquine are represented by green dots. Blue dots represent the placebo group. QTcF: Fridericia-corrected QT interval; MCS: morphology combination score

TABLE 2 Concentration-dependent effect of hydroxychloroquine on slope estimate of different electrocardiogram characteristics

	Slope estimate (per ng/mL)	Standard error	95% confidence interval	P-value
RR interval (ms)	-0.341	0.255	-0.8410 to 0.1600	.091
PR interval (ms)	0.010	0.020	-0.0302 to 0.0496	.320
QRS interval (ms)	0.005	0.011	-0.0170 to 0.0264	.340
J-Tpc interval (ms)	-0.008	0.023	-0.0534 to 0.0368	.360
Tp-Te interval (ms)	0.008	0.013	-0.0170 to 0.0320	.270
QTcF interval (ms)	0.017	0.026	-0.0331 to 0.0679	.250
MCS	6.30×10^{-5}	1.56×10^{-4}	-0.0002 to 0.0004	.340
Asymmetry	2.59×10^{-5}	7.07×10^{-5}	-0.0001 to 0.0002	.360
Flatness	1.90×10^{-5}	7.92×10^{-5}	-0.0001 to 0.0002	.410
Notch ^a	0	N/A	0 to 0	N/A

^aNotch statistics could not be performed, since none of the ECGs displayed a notch, meaning that all values were 0. J-Tpc: J-Tpeak, corrected for RR interval; Tp-Te: Tpeak-Tend; QTcF: Fridericia-corrected QT interval; MCS: morphology combination score.

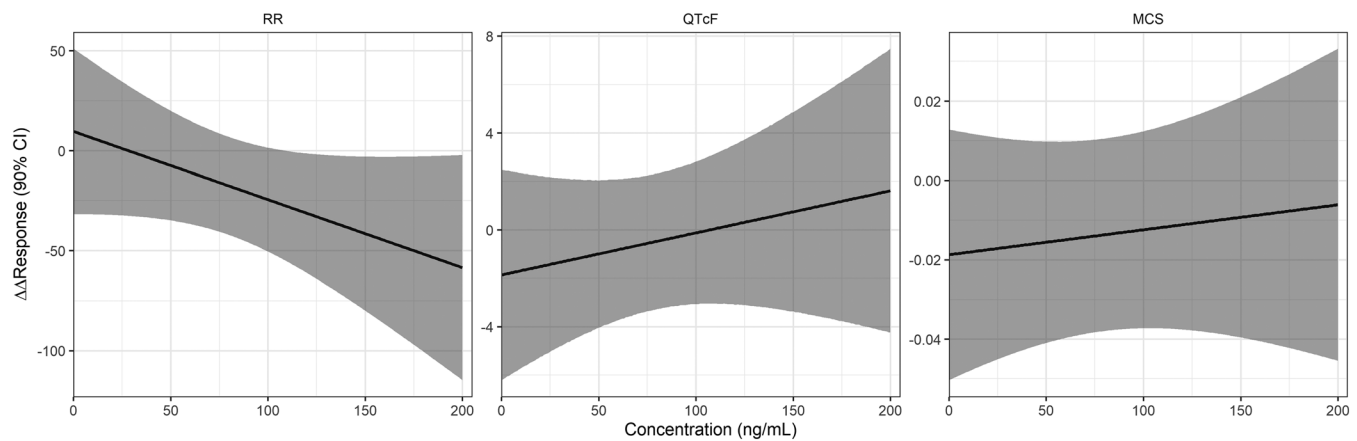


FIGURE 3 Concentration-dependent effect of hydroxychloroquine on $\Delta\Delta$ RR interval in ms (left panel), $\Delta\Delta$ QTcF interval in ms (middle panel) and $\Delta\Delta$ MCS (right panel), corrected for both baseline measurements and placebo. The grey area represents the 90% confidence interval (CI). QTcF: Fridericia-corrected QT interval; MCS: morphology combination score

flatness of the T-wave did not change (slope estimate $\Delta\Delta$ asymmetry = 2.59×10^{-5} , 95%CI -10^{-4} to 2×10^{-4} , $P = .36$; slope estimate $\Delta\Delta$ flatness = 1.90×10^{-5} , 95%CI -10^{-4} to 2×10^{-4} , $P = .41$) and there were no T-wave notches observed in any of the ECGs. As expected based on the previous results, there was no significant effect of hydroxychloroquine on the MCS ($P = .34$), as can also be observed in Figures 3 and S4. Lastly, no significant effect of age group on any of the ECG characteristics was observed.

4 | DISCUSSION

The present analysis investigated the effects of hydroxychloroquine on the ventricular repolarization with a concentration-effect analysis. No effect of hydroxychloroquine was observed on any of the studied ECG characteristics, including the QTcF-interval and T-wave morphology. This implies that, based on this analysis, the hydroxychloroquine treatment of SARS-CoV-2 patients is not expected to have led to QT-interval prolongation nor other significant changes of cardiac repolarization.

QT-interval prolongation induced by hydroxychloroquine and other QT-prolonging drugs is thought to be mediated through I_{Kr} -blockade, which is encoded by the hERG channel.^{38,39} QT-interval prolongation, which is associated with ventricular arrhythmias, has been reported as side effect of hydroxychloroquine in studies in both SARS-CoV-2 patients and patients with other conditions.^{19,40-42} Previous studies found a prolongation of the QTcF-interval after hydroxychloroquine administration from 424.4 ± 29.7 ms at baseline to 432.0 ± 32.3 ms in rheumatological patients,¹⁹ and from 416 (interquartile range [IQR] 404-433) to 423 (IQR 405-438) ms in SARS-CoV-2 patients.⁴⁰ Another study in SARS-CoV-2 patients reported an increase of 13 (IQR 9-16) ms.⁴¹ Other studies reported QTcF-prolongation after hydroxychloroquine treatment only in a minority of their cases.^{43,44} For example, an average increase of the QTcF-interval

of 1.8% was observed in a cohort of 219 hospitalized and ambulatory SARS-CoV-2 patients who were treated with a total dose of 2800 mg hydroxychloroquine, although combined with other antiviral drugs.⁴⁰ In another cohort of 111 hospitalized SARS-CoV-2 patients who were treated with a hydroxychloroquine dose similar to our study (2400 mg), only 5 (7%) of the patients developed a QTcF-prolongation.⁴⁴ Although hydroxychloroquine recently has been studied extensively, all aforementioned studies are observational and none of them use pharmacokinetic data to evaluate hydroxychloroquine's QT-prolonging effect with a concentration-effect analysis. The current randomized controlled trial in healthy subjects enables evaluation of the effect of hydroxychloroquine without confounding factors that are inherent to hospitalized patients. In critically ill patients, there are many factors that can affect ventricular repolarization and thereby the QT-interval, such as comedication, fever and comorbidities. This complicates discriminating the effect of hydroxychloroquine from other factors affecting ventricular repolarization. The performed concentration-effect analysis is needed to draw conclusions on the association between solely hydroxychloroquine and the ECG.

In the present analysis, we studied the concentration-dependent effects of hydroxychloroquine on QTcF-interval and other ECG characteristics in healthy volunteers. We found no effect of hydroxychloroquine on any of the investigated indices of ventricular repolarization, with a mean $\Delta\Delta$ QTcF at the highest hydroxychloroquine concentration that was investigated below the threshold of concern of 5 ms.²³ Additionally, there was no effect of hydroxychloroquine on the MCS (slope estimate $\Delta\Delta$ MCS = 6.30×10^{-5} , $P = .34$). As a reference, in a large primary care population (over 200 000 subjects), an increased MCS of 0.10 was associated with an increased mortality (adjusted hazard ratio for men: 1.61).⁴⁵ In our study, healthy subjects received a total dose of 2400 mg hydroxychloroquine, with a maximum plasma concentration exceeding 200 ng/mL. The half-maximum inhibitory concentration (IC₅₀) of hydroxychloroquine for hERG-channel inhibition is estimated between 2.5 and 8.4 μ M.^{38,39} Considering a 30-40%⁴⁶ protein binding of hydroxychloroquine and taking

the lowest IC₅₀ into account, this IC₅₀ is equivalent to a plasma concentration of around 840 ng/mL, >4× times higher than the maximum plasma concentration in our study.⁴⁶ As we used a standard dosing regimen for SARS-CoV-2 patients, it is considered unlikely that in hospitalized patients with comparable regimens, concentrations would be substantially higher as compared to the population in this analysis. Some regimens used higher doses of hydroxychloroquine for treatment of SARS-CoV-2 patients, but even in case of a double plasma concentration compared to the studied regimen, clinically relevant hERG channel inhibition will not be achieved as described above. Note that hydroxychloroquine has a long half-life (between 32 and 50 days), which means that impaired excretion is not expected to result in substantially greater accumulation of hydroxychloroquine in patients as compared to healthy volunteers.⁴⁷ Also, a physiologically-based (PB) PK model that investigated comparable dosages as compared to this analysis predicted a concentration that was slightly below the plasma concentrations observed in this analysis.³ Therefore, plasma concentrations are unlikely to have exceeded the measured concentrations in our study. This implies that based on this analysis, the hydroxychloroquine treatment of SARS-CoV-2 patients is not expected to have led to QTcF-prolongation by hERG-channel inhibition, as concentrations were too low to reach this effect. Moreover, comparing the group of young and elderly subjects, even in older patients, who are at risk for cardiac complications, hydroxychloroquine did not prolong the QTcF-interval in our study.

We hypothesize that the increased QT-interval duration observed in hospitalized SARS-CoV-2 patients may have been related to their health status and medication that was administered apart from hydroxychloroquine, particularly as SARS-CoV-2 patients have longer QTcF-intervals than the normal population due to cardiac involvement.⁴⁸ For example, hydroxychloroquine was often combined with azithromycin, which is independently associated with QT-prolongation and TdP.^{18,49–51} In a systematic review of 25 studies including both SARS-CoV-2 patients and rheumatic patients, hydroxychloroquine reduced the risk of cardiac events and no cases of death due to TdP were reported.⁵² Notably, in a study of 59 SARS-CoV-2 patients, no correlation was found between hydroxychloroquine plasma concentrations and body temperature.⁵³

This analysis reinforces the importance of concentration–effect analyses in assessing the effects of drugs on the ventricular repolarization. Studies investigating this association are substantially more accurate in determining these effects as compared to retrospective analyses of hospitalized patients, which typically have comorbidities and use concomitant medication affecting the QT-interval.

5 | LIMITATIONS

Although this study enabled us to analyse the effect of hydroxychloroquine on ECG characteristics profoundly, the study also had

its limitations. First, no suprathreshold dose levels were investigated. We do not expect higher plasma levels in patients as compared to healthy subjects due to the pharmacokinetic properties of this drug (i.e. high bioavailability and slow elimination).⁵⁴ However, investigation of suprathreshold doses is essential in the evaluation of QT-effects and addition of suprathreshold dose levels would have reinforced the results of this study. Second, all postdose time points only included single ECGs, while triplicate ECGs are the industry standard, and the number of hydroxychloroquine plasma concentration measurements with a matching ECG was limited to 4 per subject. Due to these constraints in study design, uncertainty of our results may have increased. Also, ECG measurements were performed automatically instead of manually or manually adjudicated measurements, which is considered the industry standard and recommended by the US Food and Drug Administration. However, the small CIs around the measured ECG parameters show that variability in our results is limited, potentially due to the larger sample size in this study as compared to typical QT waiver studies. Despite these limitations, which could have increased the level of uncertainty in the estimates, the size of the 90% CIs of the ECG parameters were still below the threshold of concern. Lastly, there was no positive control in this study. However, again the small variability in measured QT-times shows the low uncertainty and consistency in results, alleviating the need of a positive control.

6 | CONCLUSION

This concentration–effect analysis in healthy subjects provides insight in the effects of hydroxychloroquine on ventricular repolarization, without confounding factors due to illness or hospitalization. The results of this concentration–effect ECG analysis showed that hydroxychloroquine did not affect the ventricular repolarization, including QTcF-interval and T-wave morphology, at concentrations similar to concentrations achieved in SARS-CoV-2 patients. Therefore, we hypothesize that the observed QT-prolongation in SARS-CoV-2 patients is caused by other factors than their hydroxychloroquine use, although hydroxychloroquine involvement cannot be fully excluded. These results suggest that hydroxychloroquine does not increase the risk of QT-mediated ventricular arrhythmias in the studied dosing regimen and population.

DISCLOSURE

The results presented in this paper are not under consideration for publication elsewhere, and have not been published previously in whole or in part, except in abstract form. All authors declare that they have no relevant financial interests or disclosures to report.

PATIENT CONSENT

The study was approved by the Independent Ethics Committee of the Foundation *Evaluation of Ethics in Biomedical Research* (Stichting Bedoording Ethiek Biomedisch Onderzoek), Assen, The Netherlands.

CLINICAL TRIAL REGISTRATION

The study was registered in the Toetsingonline Registry under study number NL73816.056.20, and in the trialregister.nl registry under number NL8726.

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CONTRIBUTORS

B.E.M. and P.G. wrote the manuscript; B.E.M., M.J., M.M. and P.G. designed the research; B.E.M., A.i.V., M.J. and M.M. performed the research; C.G., J.K., M.v.E. analysed data and all authors reviewed and provided critical content to the manuscript.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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