ARTICLE

Chloroquine and hydroxychloroquine provoke arrhythmias at concentrations higher than those clinically used to treat COVID-19: A simulation study

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Abstract

The risk of fatal arrhythmias is the major concern for using chloroquine (CQ) or hydroxychloroquine (HCQ) to treat coronavirus disease 2019 (COVID-19), but the reported number of life-threatening arrhythmic events or deaths is relatively small. The objective of this study was to assess the arrhythmogenic risk of these two drugs using a multiscale heart simulation, which allows testing even at high concentrations, including those that cause fatal arrhythmias. We measured the inhibitory action of CQ, HCQ, and HCQ with 30 μM azithromycin (AZ) on six ion currents (fast [INa] and late [INa,L] components of the sodium current, L-type calcium current [ICa,L], rapid [IKr/hERG], and slow [IKs] components of delayed rectifier potassium, and inward rectifier potassium [IK1]) over a wide range of concentrations using the automated patch-clamp system. Using the concentration–inhibition relationship that was thus obtained, we simulated the drug effects while increasing the concentration until the life-threatening arrhythmia, torsade de pointes (TdP), was observed. The obtained threshold concentrations for TdP were 12.5, 35, and 22.5 μM for CQ, HCQ, and HCQ with AZ, respectively. Adding therapeutic concentrations of mexiletine or verapamil successfully prevented the occurrence of TdP, and verapamil was more effective. CQ, HCQ, and HCQ with AZ thresholds for TdP were larger than both antiviral concentrations that were reported by in vitro experiments and free plasma concentrations that were attained by the clinically used dosage. The current simulation data provided a safety margin to the currently used clinical dose for CQ and HCQ/AZ.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Despite the potent in vitro antiviral effect, clinical trials have failed to show the therapeutic effects of chloroquine (CQ) and hydroxychloroquine (HCQ)/azithromycin (AZ) to treat coronavirus disease 2019. Torsadogenic potentials may limit the dosage of these drugs, but the reported incidence of fatal arrhythmias is rare.

WHAT QUESTION DID THIS STUDY ADDRESS?
Our objective was to assess the arrhythmogenicity of CQ and HCQ/AZ over a wide range of drug concentrations using a multiscale heart simulation.
WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
Our study showed that CQ and HCQ/AZ do not induce fatal arrhythmias even at concentrations much higher than in vitro antiviral half-maximal effective concentration (EC$_{50}$) values at which QT prolongation exceeds 150 ms. We also found that estimated free plasma concentrations of CQ and HCQ/AZ achieved by currently used dosing protocols are lower than the antiviral EC$_{50}$ for these drugs.
HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
Our simulation data provided a safety margin to the currently used clinical dose for CQ and HCQ/AZ.

INTRODUCTION

Because of the worldwide health crisis that is caused by the coronavirus disease 2019 (COVID-19) pandemic, rigorous research is now taking place for drug compounds that are effective against this novel virus (severe acute respiratory syndrome-coronavirus 2 [SARS-CoV-2]). To date, however, the effectiveness of only a few compounds has been confirmed by clinical trials and many others have failed to show therapeutic and/or prophylactic effects. Among them, antimalarial drugs chloroquine (CQ) and hydroxychloroquine (HCQ) were considered to be promising because of the in vitro inhibitory effect of these drugs on SARS-CoV-2, which was reported to be comparable to that of remdesivir, which is one of the few clinically proven drugs,[1] but none of the large-scale clinical trials performed in many countries showed the clinical benefits of CQ and HCQ with or without the co-administration of azithromycin (AZ).[2–4] Rather, doctors and regulatory agencies are concerned about the risk of fatal arrhythmia resulting from the QT interval prolongation that is induced by these compounds and the possible increased risk with the co-administration of AZ.[5–7]

The reason for the discrepant results on the efficacy between in vitro experiments and clinical observations is not clear, but the consideration of the plasma concentration may be of value. Due to the arrhythmogenic risk, the dosing regimens of HCQ and CQ are conservative in the protocols that are used against malaria. However, pharmacokinetic simulations suggested that the plasma concentrations that were attained by these regimens are too low to achieve the antiviral effect that is observed in vitro.[8,9] Results of these reports can be taken to indicate that with the modifications of dosing protocol and the possible use of a new drug delivery system, with which high enough tissue concentration of the drug is attained safely, the use of CQ or HCQ may be reconsidered as a therapeutic compound for COVID-19. However, testing this hypothesis in a patient population is ethically and practically unfeasible.

Computer simulations enable us to perform practically unfeasible experiments under controlled conditions, and they have already been used in biology and medicine. We have developed a multiscale, multiphysics heart simulator[10,11] and used it to evaluate drug-induced arrhythmias to show that the simulation can accurately predict the arrhythmogenic risk of drugs.[12,13] In those studies, we also showed that QT prolongation may not be a reliable biomarker that represents the arrhythmogenic risk for certain types of drugs.

Thus, the objective of this study was to predict the safe dosage to prevent the arrhythmogenic risk of CQ and HCQ with or without the co-administration of AZ. We also investigated measures to lessen the risk by adding another drug with different ionic actions. The results revealed a high threshold concentration for arrhythmogenicity, which may necessitate the reconsideration of these drugs as treatments for COVID-19.

METHODS

Similar to our previous study, we used a hybrid approach, in which inhibitory actions of drugs on ion currents were determined by patch-clamp experiments, and the results were applied to the simulator as input data.[12] The pharmacological data for CQ and HCQ were obtained using the protocol that is described below. The effect of HCQ was also tested in the presence of 30 μM AZ. This AZ concentration was markedly higher than the peak plasma concentration (C$_{max}$) observed with oral dosing in clinical use (0.22–1.1 μM).[14,15] We expect a mild-to-moderate proarrhythmic effect at this concentration. The effect of AZ around this concentration was also examined.

In vitro ion channel assay

Ion currents were recorded in Chinese hamster ovary (CHO) or Chinese hamster lung (CHL) cells expressing hERG (IKr), Nav1.5 (INa), Cav1.2/β 2/α 2-δ (ICa), KCNQ1+KCNE1 (IKs), or Kir2.1 (IK1) channels using the QPatch-II system (Sophion Bioscience, Copenhagen, Denmark) and using the cell lines, assay buffers, and voltage protocols that were described in previous studies.[12,16] Because it was not possible to monitor

the drug concentration in the experimental chamber with the QPatch system, drug concentration was instead verified in the stock solution. Maximal care was taken to avoid adsorption of the drug by coating the entire system using Teflon or glass. The late component of INa (INa-L) was induced by application of a depolarizing pulse to -15 mV for 40 ms followed by step pulse to 40 mV for 200 ms and 100 ms ramp pulse to -80 mV every 5 s. ATX-II (Alomone Labs, Jerusalem, Israel) was also used to induce INa-L. The inhibitory effect on INa-L of INa was also included using previously reported parameters.[17,18] For the accurate evaluation of arrhythmogenic risk, data should be collected at a physiological temperature. However, due to the difficulty of experiments at physiological temperature with an automated patch system, assays were performed at room temperature (25°C).[16] Only the hERG current was assayed at both room and physiological temperatures (37°C) due to its dominant role in the drug-induced arrhythmias.[19]

Drug effects were analyzed for each channel by normalizing the current by its maximum value that was obtained in the absence of the drug and fit to the Hill equation, where x is the drug concentration, h is Hill constant, and IC_{50} is the drug concentration at which 50% inhibition of the ionic current is observed.

$$\text{Relative current} = \frac{1}{1 + 10^{(\log IC_{50} - \log x) \cdot h}}$$

Nonlinear least-square fits were solved using GraphPad Prism version 8.3.1 (San Diego, CA).

Simulation of electrophysiology and electrocardiogram analysis

Simulation of drug effects on human cardiac electrophysiology was performed using the UT-Heart model of the healthy adult ventricles, and the details of this model have been reported previously.[11-13] Briefly, UT-Heart is a realistic three-dimensional heart model that is based on the finite element method (FEM), and we used cell models that were specific for the ventricular myocyte[20,21] and conduction system.[22] They were implemented to each FEM node, while reproducing the physiological distribution of different cell species (endocardial, M, and epicardial cells) in the ventricular wall.[23] The propagation of excitation was solved with the bidomain formulation using the parallel multilevel technique that we previously developed.[11] To save computational time, only the ventricles with a conduction system were modeled, and the stimulus was always applied to the root of the conduction system at 1 Hz (60 beats/min) to mimic regular sinus rhythm. We examined the effects of CQ, HCQ, and HCQ with AZ over a wide range of concentrations. Under control conditions, we ran cell models for 1000 beats to achieve steady-states and the final states of channel gates were applied to the heart

model as initial conditions. Next, we calculated the 10 beats of heart simulation, and the final states of channel gates of each FEM node were saved as initial conditions of the heart. For each condition, we performed the simulation for 5 s while pacing the ventricles at 1 Hz. Accordingly, we analyzed five beats as standard but analyzed additional beats when arrhythmias were observed. From the body surface potential data, we obtained the 12-lead electrocardiogram (ECG) based on the standard lead locations and measured the QT intervals in limb lead II. We also examined the effects of anti-arrhythmic drugs on the arrhythmogenicity of CQ, HCQ, and HCQ with AZ. To analyze the drug interactions under concomitant use of anti-arrhythmic drugs, we tested competitive and noncompetitive models (Figure S1) and found that the competitive model yielded fewer anti-arrhythmic effects. Thus, we used the competitive model to avoid overestimation of drug effects. Computation was performed using the Oakforest-PACS that was provided by the Joint Center for Advanced High Performance Computing (Intel Xeon Phi7250 [1.4 GHz], 8208 nodes) and Supercomputer Fugaku at the RIKEN Center for Computational Science (Armv8.2-A SVE [2.0 GHz], 158976 nodes). The computational times for a single beat using 2354 cores were 55 min and 35 min, respectively. All program codes were written in-house and validated in our previous studies.[12,13,23,24] Sample model code and dataset are available at Figshare (https://doi.org/10.6084/m9.figshare.13325330).

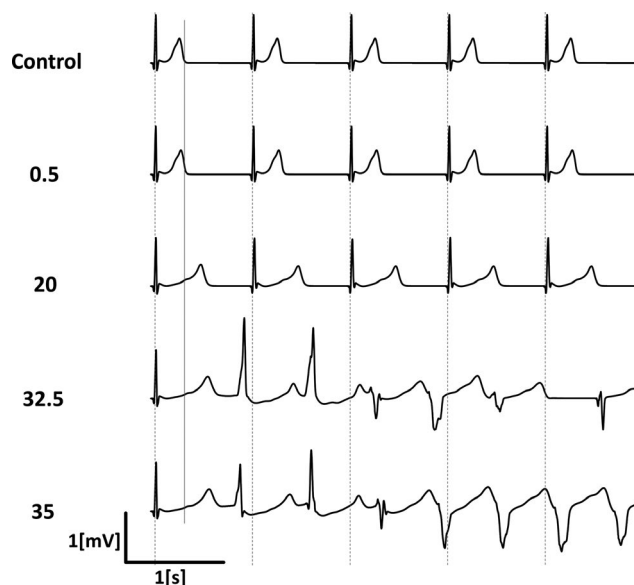## RESULTS

Drug effects on multiple ion channels

Multichannel effects of CQ, HCQ, and HCQ + AZ are summarized in Figure 1. For hERG current, data at 37°C (solid line) and 25°C (broken line) are shown. Significant inhibitions on the hERG current were observed even in the submicromolar range for both drugs, but the IC_{50} was lower for CQ (2.1 μM at 37°C, 2.7 μM at 25°C; Figure 1a) compared with HCQ (5.5 μM at 37°C, 16.6 μM at 25°C; Figure 1b). A similar tendency was observed for the IK1 current. Adding AZ to HCQ decreased the IC_{50} for the hERG current (3.78 μM at 37°C, 19.98 μM at 25°C; Figure 1c), but slightly increased the IC_{50} for IK1 and INaL. For the temperature dependence of the hERG current, lower IC_{50} leading to a higher degree of QT prolongation and arrhythmogenic risk were obtained at 37°C for all drugs. Because the purpose of this study was risk assessment, we used the hERG IC_{50} at 37°C in subsequent analyses. Regarding the effect of AZ at 37°C, we observed 14% of hERG current inhibition at 30 μM ($IC_{50} = 325.8$ μM; Figure 1d). This effect is smaller than that reported by Crumb et al.,[25] but may have contributed to the small shift in hERG IC_{50} of HCQ induced by the addition of AZ (5.5 to 3.78 μM). The reason for the discrepant results between the Crumb et al. report and the present study is not clear, but

FIGURE 1 Drug effects on multiple ion currents. (a) Concentration-dependent inhibitory action of chloroquine (CQ) on six ion currents. (b) Concentration-dependent inhibitory action of hydroxychloroquine (HCQ) on six ion currents. (c) Concentration-dependent inhibitory action of HCQ with 30 μM azithromycin (AZ) on 6 ion currents. (d) Effect of AZ on multiple ion currents. The assay was performed in the concentration range of approximately 30 μM. Bars indicate SD ($N = 6$ for each data point)
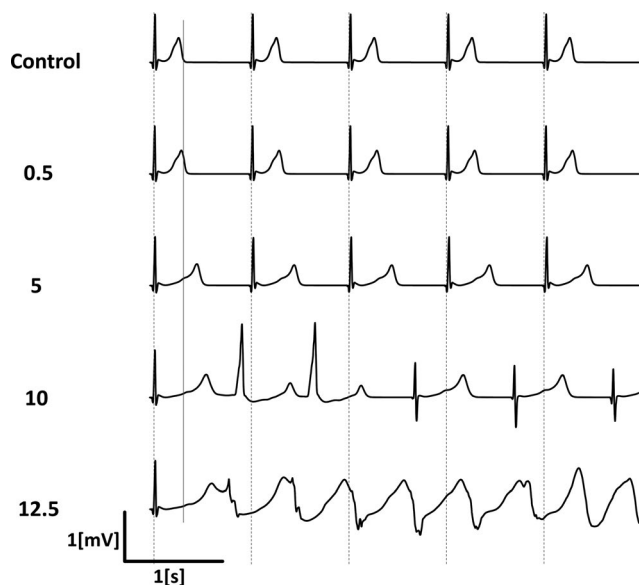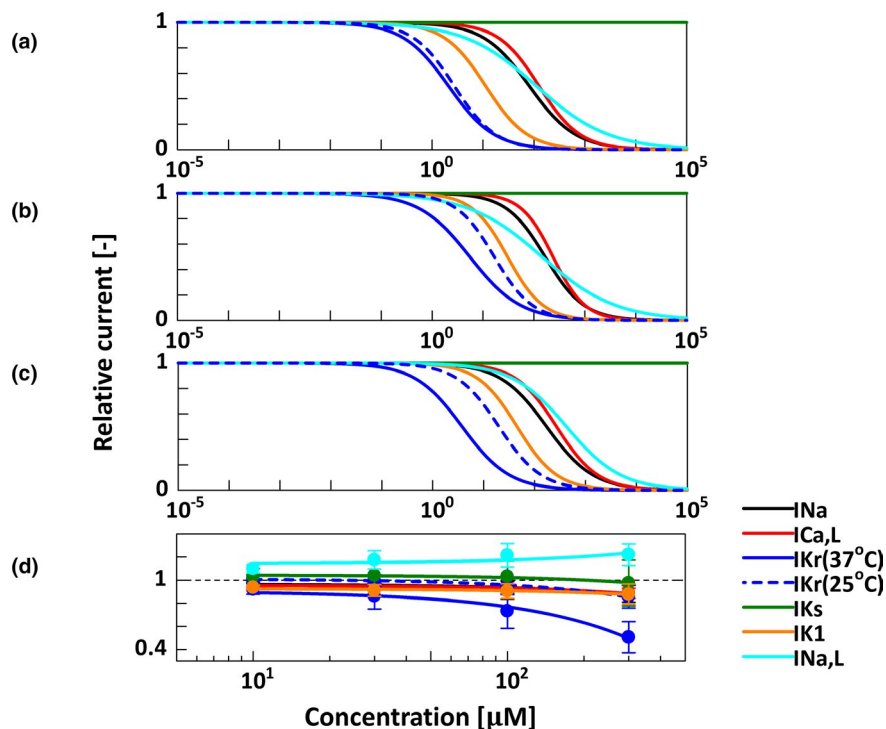
FIGURE 2 Electrocardiogram (ECG) changes induced by varying the chloroquine (CQ) concentration. Second-limb lead ECGs are shown for increasing CQ concentrations. Numbers to the left of each panel indicate concentrations in μM. Control, without drug

FIGURE 3 Electrocardiogram (ECG) changes induced by varying the hydroxychloroquine (HCQ) concentration. Second-limb lead ECGs are shown for increasing chloroquine (CQ) concentrations. Numbers to the left of each panel indicate concentrations in μM. Control, without drug

differences in experimental condition may have been a factor. We also found a small but significant increase in INaL, consistent with an earlier report.[15] Although the magnitude of increase was small, INaL activation may additively contribute to prolongation of QT interval caused by hERG current inhibition by AZ. Finally, differences in experimental settings between HCQ + AZ and HCQ alone should be considered. IC$_{50}$ and Hill constant values are summarized in Supplementary Table S1.
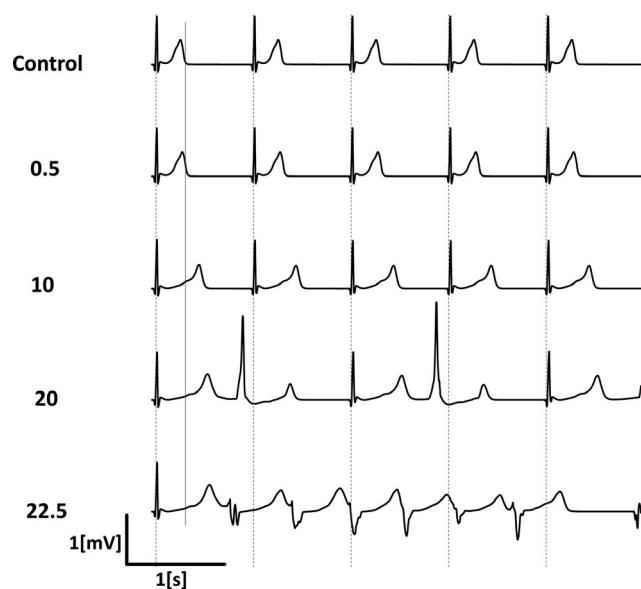
Arrhythmogenicity of drugs

Using the pharmacological data shown above, we performed heart simulations while changing the drug concentrations. CQ prolonged the QT interval in a concentration-dependent manner and, unexpectedly, the prolongation (ΔQT) increased to 186 ms at 5 μM without causing arrhythmias (Figure 2).

Arrhythmias first appeared at 10 µM, and at 12.5 µM we observed torsade de pointes (TdP). Similar observations were made with HCQ, but the threshold concentrations for ventricular arrhythmias and TdP were higher (32.5 and 35 µM; Figure 3). Co-administration of AZ augmented the risk by lowering the threshold for ventricular arrhythmias (32.5–20 µM) and TdP (35–22.5 µM; Figure 4). Co-administration of AZ prolonged the QT interval compared with HCQ alone (35 ms vs. 27 ms at 1 µM HCQ), which are attributable to the reduction in the hERG IC_{50} and activation of INaL.

Risk reduction by anti-arrhythmic drugs

We studied the effect of two anti-arrhythmic drugs, mexiletine and verapamil, on the arrhythmogenicity of CQ, HCQ, and HCQ + AZ. Mexiletine was examined to determine whether it can counteract QT prolongation associated with hERG block[26] and mitigate TdP events.[27] Verapamil was selected given that we identified a reasonably potent anti-arrhythmic effect by multichannel blockade on ICaL and hERG in our previous study.[13] Pharmacological properties and effective free plasma concentrations of these drugs were taken from previously published studies.[12,25] Mexiletine prevented TdP that was induced by 12.5 µM of CQ but only at a high concentration (4-times of effective therapeutic plasma concentration; Figure 5a, third row). However, verapamil was effective even at the level of the effective therapeutic plasma concentration, but inhibition of sodium current by CQ prolonged the QRS width (Figure 5a, fourth row). The
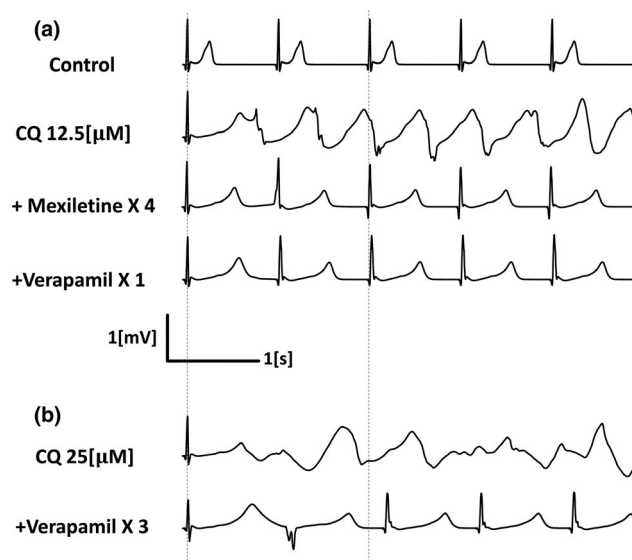
therapeutic concentration of verapamil was also effective for TdP that was induced by a higher concentration of CQ, which mexiletine failed to terminate (Figure 5b). For TdP that was induced by HCQ, mexiletine exerted an effect at four-times the effective therapeutic plasma concentration (Figure 6, third row), but under this condition, heart rate was slightly faster than 1 Hz. Further examination revealed that this was attributable to the automaticity of the conduction system (Figure S2). Although the intrinsic rate of automaticity of the conduction system is slower than 1 Hz, elevated resting membrane potential evoked by the inhibitory action on the IK1 current of HCQ allows the membrane potential to more rapidly reach the threshold potential for triggering the action potential. With HCQ alone, this effect was masked by the appearance of arrhythmias but became apparent when arrhythmias were inhibited by mexiletine or verapamil. Verapamil was very effective (Figure 6a, fourth row). Finally, two anti-arrhythmic drugs were also effective for TdP that was induced by 22.5 µM HCQ with 30 µM AZ (Figure 6b).

DISCUSSION

In this simulation study using a healthy heart model, we found that CQ and HCQ with or without the co-administration of AZ induced QT prolongation in a dose-dependent manner and provoked TdP at high concentrations. However, the

FIGURE 4 Electrocardiogram (ECG) changes induced by varying the concentration of hydroxychloroquine (HCQ) with 30 µM azithromycin (AZ). Second limb lead ECGs are shown for an increasing concentration of chloroquine (CQ). Numbers to the left of each panel indicate the concentrations in µM. Control, without drug

FIGURE 5 The effect of antiarrhythmic drugs on the arrhythmogenicity of chloroquine (CQ). (a) From the top: electrocardiogram (ECG) without drug, ECG with 12.5 µM CQ, ECG with 12.5 µM CQ and 4 times the therapeutic free plasma concentration of mexiletine, ECG with 12.5 µM CQ, and the therapeutic free plasma concentration of verapamil. (b) Top row: ECG with 25 µM CQ. Bottom row: ECG with 25 µM CQ and 3 times the therapeutic free plasma concentration of verapamil
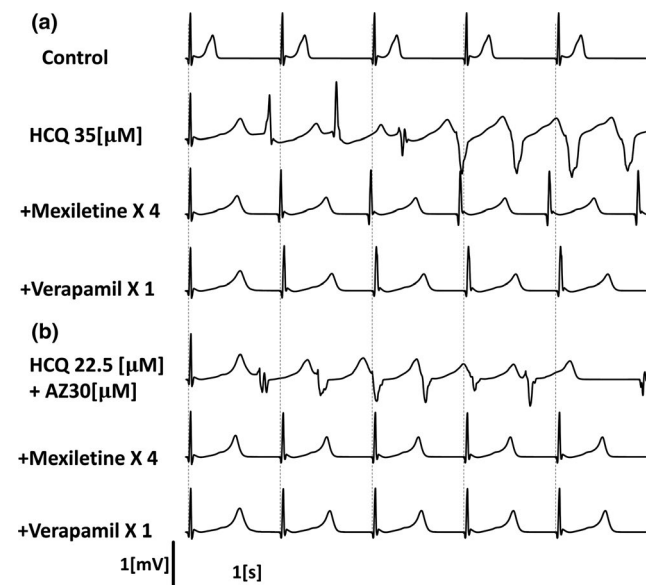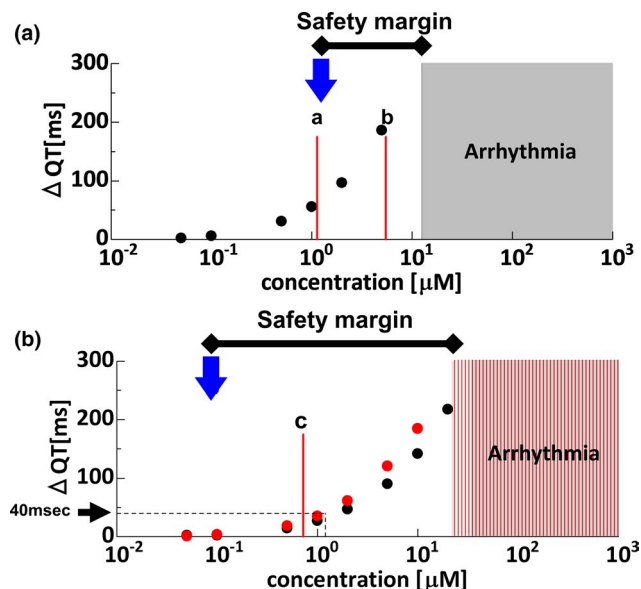
FIGURE 6 The effect of antiarrhythmic drugs on the arrhythmogenicity of hydroxychloroquine (HCQ). (a) Starting at the top: Electrocardiogram (ECG) without drug, ECG with 35 μM HCQ, ECG with 35 μM HCQ and 4 times the therapeutic free plasma concentration of mexiletine, and ECG with 35 μM HCQ and the therapeutic free plasma concentration of verapamil. (b) The effect of antiarrhythmic drugs on the arrhythmogenicity of HCQ with 30 μM azithromycin (AZ). From the top: ECG with 22.5 μM HCQ with 30 μM AZ, ECG with 22.5 μM HCQ with 30 μM AZ and 4 times the therapeutic free plasma concentration of mexiletine, and ECG with 22.5 μM HCQ with 30 μM AZ and the therapeutic free plasma concentration of verapamil

FIGURE 7 Relationship between QT prolongation and therapeutic concentrations of drugs. (a) The relationship between QT prolongation (ΔQT) and chloroquine (CQ) concentration (black circles) is shown with the therapeutic free plasma concentration that was estimated using the physiologically-based pharmacokinetic (PBPK) model (blue arrow)[29] and the anti-viral half-maximal effective concentration (EC$_{50}$) measured *in vitro* (red lines: a, by Wang et al.[1]; b, by Yao et al.[29]). The gray rectangle indicates the region of the arrhythmia. (b) The relationship between QT prolongation (ΔQT) and hydroxychloroquine (HCQ; black circles) or HCQ with 30 μM azithromycin (AZ; red circles) concentrations are shown with the therapeutic free plasma concentration that was estimated using the PBPK model (blue arrow)[8] and the antiviral EC$_{50}$ that was measured in vitro by Yao et al. (red line).[29] Gray and red rectangles indicate the region of arrhythmia for HCQ and HCQ with 30 μM AZ. The broken line indicates the concentration of HCQ + AZ that induces a ΔQT of 40 ms

threshold concentrations for arrhythmias were higher than the clinical C$_{max}$ associated with doses causing arrhythmias in prior clinical studies. We also found that co-administration of the commonly used anti-arrhythmic drugs, mexiletine and verapamil, can increase the threshold concentrations for arrhythmias.

Dosing regimens and antiviral effect

Despite the potent antiviral effect that was observed in vitro, clinical trials involving a large number of patients have failed to show the benefit of CQ or HCQ with or without AZ.[2-4] The reason for these disappointing results is not clear, but we speculated that the difference in the drug concentrations between the experiments and clinical settings, at least in part, can be a factor. Wang et al., using Vero E6 cells that were infected with nCoV-2019/Wuhan/WiV04/2019, evaluated the antiviral effects of drugs by quantifying the viral copy number in the cell supernatant after 48 h of incubation to find that the half-maximal effective concentration (EC$_{50}$) of CQ is 1.13 μM, which is comparable with that of remdesivir (0.77 μM).[1] These authors reported higher values (2.71, 3.81,

7.14, and 7.36 μM at multiplicity of infections of 0.01, 0.02, 0.2, and 0.8, respectively). Using a similar assay system, Yao et al. reported the EC$_{50}$ of CQ and HCQ to be 5.47 μM and 0.72 μM, respectively.[29]

However, in the dosing protocols that were used in clinical trials, HCQ was given at a dose of 600 mg twice on day 1, then 400 mg daily for a median of 5 days,[2] or as a loading dose of 1200 mg daily for 3 days followed by a maintenance dose of 800 mg daily for 2 or 3 weeks.[4] CQ was given as 600 mg twice daily for 10 days or 450 mg twice daily on the first day and 450 mg once daily for 4 days.[3] A simulation study using a physiologically-based pharmacokinetic (PBPK) model of adults reported an average free plasma concentration of 32 ng/ml, which corresponds to 0.095 μM, when the drug was given as 400 mg every 12 h on day 1, followed by 200 mg every 12 h for 4 days.[8] For CQ, another PBPK study reported a plasma concentration of about 400 ng/ml (1.25 μM) with a dose of 500 mg twice

daily for 10 days.[29] Because the authors did not mention whether this value was free or total plasma concentration, the free plasma concentration of CQ could be as low as half of this value. These data are summarized in Figure 7 with the QT prolongation affects that were obtained in this study. Although the free plasma CQ concentration under the clinically used dosing protocol (blue arrow) could reach the lower range of antiviral concentration (red vertical line in Figure 7a), the estimated therapeutic free plasma concentration of HCQ with or without AZ (blue arrow) was less than the antiviral concentration (red vertical line in Figure 7b). In either case, the safety margin for the region of arrhythmias is large, suggesting the possibility of increasing the dose. Free plasma concentrations in clinical settings can be estimated using an alternative approach. Chorin et al. examined the ECG changes in patients who were treated with HCQ and AZ for 5 days (HCQ: 800 mg on the first day followed by 400 mg daily; AZ: 500 mg daily), and they found that the average prolongation of QTc (ΔQTc) was 28 ms and less than 40 ms in 55% of the patients.[6] If we plot this ΔQTc value in the concentration–ΔQTc relationship in Figure 7b, the free plasma concentration is estimated to be below 1 μM in about half of the patient population (indicated by the broken line in Figure 7b). This value is close to the result of the PBPK simulation, and it leaves a sufficient safety margin (Figure 7b broken line). Additionally, co-administration of mexiletine or verapamil could widen the safety margins.

Is the QT interval a reliable biomarker?

A major concern with the use of CQ of HCQ is the arrhythmogenicity of these drugs. In a study that enrolled 90 patients treated with HCQ with or without AZ, 3% of patients receiving monotherapy showed a change in QTc greater than or equal to 60 ms and 13% of those treated with concomitant AZ had a change in QTc greater than or equal to 60 ms.[7] Among the patients treated with HCQ + AZ, one patient discontinued treatment because of QTc prolongation and developed TdP 3 days later. Similarly, in another study on patients who were treated with HCQ and AZ, 12% had a change in QTc of 60 ms or more. However, no TdP events were reported for any patient.[6] The absence of TdP events in the study by Chorin et al. could be attributable to the lower dose of HCQ compared with that used by Merculo et al.[7] (200 mg vs. 400 mg on days 2–5). Our results are also consistent with another study that reported no drug-induced life-threatening arrhythmia or death.[30] In our previous study, we showed that the prolongation of QT interval may not be a reliable biomarker of arrhythmogenic risk for certain drugs.[12] Given that the present data show that TdP was not observed with a ΔQT of 200 ms or longer, it may be worth reconsidering the

permissible range of QT prolongation, especially in patients without heart disease.

Study limitations

There are several limitations in this study. First, due to the difficulty of conducting experiments at physiological temperature with an automated patch-clamp system, only the hERG current was assayed at a physiological temperature, but other channels should also be assayed similarly. Additionally, due to the technical difficulty, drug inhibition on the peak current was assayed, but the effect on the kinetics needs to be studied in detail.[31] Uncertainty in the protein binding used to calculate free C_{max} also should be taken into account. Second, although the patient population is expected to be heterogeneous, including those with risk factors for arrhythmias, such as hyperkalemia and genetic propensity for arrhythmias, we used only the healthy heart model, which lacks the re-entry pathways. Furthermore, although simulations were performed while regularly paced at 60 beats/min, TdP is usually associated with slower heart rate and observed after a long-short-long beat interval pattern. Thus, the risk may have been underestimated. Furthermore, the scope of the research should be broadened to the higher temperature because most of the patients with COVID-19 are febrile. A high temperature has been reported to affect the drug effect on the channels[32] and channel properties.[33] Significant variability in the ΔQTc response among the patients[6] can be explained by the factors discussed above. All these issues need to be addressed in a future study.

CONCLUSIONS

Using the multiscale heart simulation coupled with the in vitro ion current assay with the automated patch-clamp system, we evaluated the arrhythmogenicity of CQ and HCQ with or without AZ. These drugs induce QT prolongation in a dose-dependent manner and provoke TdP at high concentrations, which may be higher than C_{max} associated with clinically relevant doses.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

J.O. and S.S. wrote the manuscript. J.O., S.S., K.S., and T.H. designed the research. J.O. and T.Y. performed the research. J.O., T.Y., and S.S. analyzed the data. J.O. and T.W. contributed new analytical tools.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.