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COVID-19 Drugs Chloroquine and Hydroxychloroquine, but Not Azithromycin and Remdesivir, Block hERG Potassium Channels

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ABSTRACT

Drug-induced long QT syndrome (LQTS) is an established cardiac side effect of a wide range of medications and represents a significant concern for drug safety. The rapidly and slowly activating delayed rectifier K^+ currents, mediated by channels encoded by the human ether-a-go-go–related gene (*hERG*) and KCNQ1 + KCNE1, respectively, are two main currents responsible for ventricular repolarization. The common cause for drugs to induce LQTS is through impairing the hERG channel. For the recent emergence of COVID-19, caused by severe acute respiratory syndrome coronavirus 2, several drugs have been investigated as potential therapies; however, there are concerns about their QT prolongation risk. Here, we studied the effects of chloroquine, hydroxychloroquine, azithromycin, and remdesivir on hERG channels. Our results showed that although chloroquine acutely blocked hERG current (I_{hERG}), with an IC_{50} of 3.0 μM, hydroxychloroquine acutely blocked I_{hERG} 8-fold less potently, with an IC_{50} of 23.4 μM. Azithromycin and remdesivir did not acutely affect I_{hERG}. When these drugs were added at 10 μM to the cell culture medium for 24 hours, remdesivir increased I_{hERG} by 2-fold, which was associated with an increased mature hERG channel expression. In addition, these four drugs did not acutely or chronically affect KCNQ1 + KCNE1 channels. Our data provide insight into COVID-19 drug-associated LQTS and cardiac safety concerns.

SIGNIFICANCE STATEMENT

This work demonstrates that, among off-label potential COVID-19 treatment drugs chloroquine, hydroxychloroquine, azithromycin, and remdesivir, the former two drugs block hERG potassium channels, whereas the latter two drugs do not. All four drugs do not affect KCNQ1 + KCNE1. As hERG and KCNQ1 + KCNE1 are two main K^+ channels responsible for ventricular repolarization, and most drugs that induce long QT syndrome (LQTS) do so by impairing hERG channels, these data provide insight into COVID-19 drug-associated LQTS and cardiac safety concerns.

Introduction

The pandemic of the novel coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), represents an unprecedented healthcare challenge. Given the urgently needed but limited treatment options for COVID-19, off-label use of chloroquine and hydroxychloroquine, sometimes with azithromycin, is under investigation (Kamp et al., 2020).The antiviral drug remdesivir is being actively investigated as well (Spinner et al., 2020). Although the effectiveness of these drugs has been reported in various clinical trials (Gautret et al., 2020; Monti et al., 2020), concerns about their proarrhythmic effects associated with prolonged QT intervals have been raised (Ramireddy et al., 2020; Mercuro et al., 2020; Hooks et al., 2020; Gérard et al., 2020; Szekely et al., 2020; Maraj et al., 2020; Chorin et al., 2020).

An abnormal prolongation of QT interval in ECG is defined as long QT syndrome (LQTS), which can develop into fatal ventricular arrhythmia torsade de pointes and lead to sudden cardiac death (Schwartz et al., 1993; Curran et al., 1995; Roden et al., 1996; January et al., 2000; Keating and Sanguinetti, 2001). Inherited LQTS occurs in 1 out of ~2000 individuals as a result of mutations in various ion channels and adaptor proteins in the heart (Schwartz et al., 2009, 2012; Zaklyazminskaya and Abriel, 2012). When LQTS occurs because of an environmental stressor, such as specific drug usage or hypokalemia, it is termed acquired LQTS. Drug-induced LQTS is a side effect of a surprisingly wide spectrum of medications; it represents the single most common cause of the withdrawal or restriction of the use of prescription drugs (Roden, 2004), and it is a significant concern during drug development, as many lead compounds with therapeutic potential are dropped from further development because of this risk (Finlayson et al., 2004; Shah, 2005). Although many types of ion channels contribute to the cardiac action potential,

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ABBREVIATIONS: COVID-19, coronavirus disease 2019; CTL, control; HEK, human embryonic kidney; hERG, human ether-a-go-go–related gene; I_{hERG}, hERG current; $I_{KCNQ1+KCNE1}$, KCNQ1 + KCNE1 current; I_{Kr}, rapidly activating delayed rectifier K^+ current; I_{Ks}, slowly activating delayed rectifier K^+ current; KCNE1, Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 1; KCNQ1, Potassium Voltage-Gated Channel Subfamily Q Member 1; LQTS, long QT syndrome; MEM, Minimum Essential Medium; QTc, corrected QT interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TBST, Tris-buffered saline with 1% Tween 20; $V_{1/2}$, voltage of half-maximal activation.

most drugs that cause LQTS do so by acting on the cardiac rapidly activating delayed rectifier K^+ current (I_{Kr}) (Roden et al., 1996; Mitcheson et al., 2000; January et al., 2000; Keating and Sanguinetti, 2001; Roden, 2004; Perrin et al., 2008), which is conducted through channels encoded by human ether-a-go-go–related gene (*hERG*) (Sanguinetti et al., 1995; Trudeau et al., 1995; Sanguinetti and Tristani-Firouzi, 2006). Experimentally, the current of hERG channels expressed in cell lines remarkably resembles I_{Kr} in the cardiac myocytes (Guo et al., 2007, 2009). hERG current (I_{hERG}) is critical for repolarization of the cardiac action potential (Curran et al., 1995; Keating and Sanguinetti, 2001). A reduction of I_{hERG} prolongs ventricular action potential duration, which is the cellular basis for QT intervals in ECG recordings.

Chloroquine has been used for malaria treatment and prophylaxis (Coban, 2020). During the severe acute respiratory syndrome pandemic in 2002–2004, chloroquine was reported to inhibit severe acute respiratory syndrome coronavirus (Keyaerts et al., 2004). It has been added to the list of trial drugs for the treatment of COVID-19 (Hu et al., 2020). Hydroxychloroquine is a derivative of chloroquine with an additional hydroxyl group. It is less toxic than chloroquine and has been used to treat autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis, and it is also considered as a candidate drug to treat COVID-19 (Liu et al., 2020). Experimentally, both chloroquine and hydroxychloroquine are reported to efficiently inhibit SARS-CoV-2 infection in vitro (Liu et al., 2020). A clinical trial indicated an association of hydroxychloroquine treatment with viral load reduction/disappearance in patients with COVID-19, and this association is reinforced by the addition of the macrolide antibiotic azithromycin (Gautret et al., 2020). On the other hand, it was reported recently that the effectiveness of hydroxychloroquine on COVID-19 warrants further investigation (Monti et al., 2020). Nevertheless, chloroquine and hydroxychloroquine remain treatment options to fight COVID-19 (Colson et al., 2020). However, patients with COVID-19 receiving chloroquine or hydroxychloroquine as a treatment were associated with a high risk of corrected QT interval (QTc) prolongation (Ramireddy et al., 2020; Hooks et al., 2020; Gérard et al., 2020; Szekely et al., 2020), and those receiving both hydroxychloroquine and azithromycin were associated with greater changes in QTc (Chorin et al., 2020; Mercuro et al., 2020). The antiviral agent remdesivir (Veklury; Gilead Sciences) has demonstrated potent antiviral activity against SARS-CoV-2 in preclinical studies and has emerged as a drug for COVID-19 treatment (Lamb, 2020). The risk of remdesivir for inducing LQTS is unknown.

In the present study, to obtain insight into potential QT prolongation induced by COVID-19 drugs, we investigated the effects of chloroquine, hydroxychloroquine, azithromycin, and remdesivir on hERG channels stably expressed in an hERG-HEK cell line. We also examined their effects on KCNQ1 + KCNE1 channels, which mediate the slowly activating delayed rectifier K^+ current (I_{Ks}) (Sanguinetti et al., 1996; Barhanin et al., 1996). The clinical implications of our findings are discussed.

Materials and Methods

Molecular Biology. The HEK293 cell line stably expressing hERG channels (hERG-HEK cells) was provided by Dr. Craig January (University of Wisconsin, Madison) (Zhou et al., 1998b). Human KCNQ1 and KCNE1 cDNAs were provided by Dr. Michael Sanguinetti (University of Utah) (Sanguinetti et al., 1996). HEK293 cell line stably expressing KCNQ1 + KCNE1 (KCNQ1+KCNE1-HEK cells) was created using plasmid transfection followed by G418 (1 mg/ml) selection (Guo et al., 2011). hERG-HEK and KCNQ1+KCNE1-HEK cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, nonessential amino acids (1×), sodium pyruvate (1 mM), and G418 (0.4 mg/ml). For electrophysiological recordings, the cells were collected from the culture dish and kept for up to 4 hours in vials with MEM at room temperature until use.

Electrophysiological Recordings. The whole-cell voltage-clamp method was used to record I_{hERG} and KCNQ1 + KCNE1 current ($I_{KCNQ1+KCNE1}$) (Guo et al., 2011). Pipettes were pulled from thin-walled borosilicate glass (World Precision Instruments, Sarasota, CA) using a micropipette puller (P-1000; Sutter Instrument, Novato, CA) and polished using a Micro Forge (MF-830; Narishige, Tokyo, Japan) to achieve a resistance of ~2.0 MΩ when filled with solution. Axopatch 200B amplifier, Digidata 1440A digitizer, and pCLAMP10.7 software (Molecular Devices, San Jose, CA) were used for data acquisition and analysis. The pipette solution contained (in millimolar) 135 KCl, 5 EGTA, 5 MgATP, and 10 HEPES (pH 7.2 with KOH). The bath solution contained (in millimolar) 5 KCl, 135 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.4 with NaOH). Recordings were carried out at room temperature (22 ± 1°C).

Western Blot Analysis. After treatments, hERG-HEK cells were collected in ice-cold phosphate-buffered saline. The whole-protein samples were made by lysing cells using sonication in ice-cold radioimmunoprecipitation assay lysis buffer supplemented with 1 mM phenylmethylsulfonyl fluoride and 4% protease inhibitor cocktail. A DC Protein Assay Kit (Bio-Rad, Hercules, CA) was used to determine the protein concentration of samples. In total, 15 μg of protein in 50 μl Laemmli loading buffer containing 5% β-mercaptoethanol was boiled for 5 minutes and loaded on 8% SDS polyacrylamide electrophoresis gels for separation for 3 hours. Separated proteins were transferred onto polyvinylidene difluoride membranes overnight in a cold room (4°C). The membranes were blocked for 1 hour using 5% nonfat milk in Tris-buffered saline with 1% Tween 20 (TBST). The blots were probed for 1 hour with anti-hERG primary antibodies in 5% nonfat milk in TBST and then incubated with the corresponding horseradish peroxidase–conjugated secondary antibodies in TBST for 1 hour. Primary and secondary antibodies were washed three times each using TBST for 5 minutes. Detection of β-actin was performed for loading control. The blots were visualized with Fuji medical X-ray films (Minato, Tokyo, Japan) using an enhanced chemiluminescence detection kit (GE Healthcare, Little Chalfont, United Kingdom). Protein band sizes were determined by loading BLUeye Prestained protein ladder (GeneDirex, Taiwan) on each gel. The hERG channel protein displays two distinct bands at molecular masses of 135 and 155 kDa. The 135-kDa band corresponds to the immature core-glycosylated form of the channel located in the endoplasmic reticulum, whereas the 155-kDa band is the fully glycosylated form of the channel present on the plasma membrane. The 155-kDa mature form of the channel is responsible for generating I_{hERG} (Zhou et al., 1998a; Guo et al., 2007, 2009; Lamothe et al., 2016, 2018). For quantification of Western blot data in Fig. 5, B and C, band intensities of hERG proteins in each treatment were normalized to their respective actin loading control and then to the control (CTL, or 0) in the same gel and expressed as relative values.

Drugs and Reagents. Hydroxychloroquine sulfate and azithromycin were purchased from Cayman Chemical Company (Ann Arbor, MI). Chloroquine disulfate was purchased from Sigma-Aldrich (St. Louis, MI). Remdesivir was obtained from Tocris Bioscience (Bristol, UK). Hydroxychloroquine sulfate and chloroquine disulfate were diluted in double distilled water, azithromycin was diluted in ethanol, and remdesivir was diluted in DMSO to stock solutions of 10 and 50 mM and stored at −20°C. To study drug block, the drugs were diluted in bath solution and used within 8 hours for patch-clamp experiments. To study chronic drug effect, drugs were diluted in MEM at

the desired concentrations. To exclude any potential vehicle-related effects on channel activities in studies of drug-channel interaction, corresponding amounts of vehicles were applied to control groups. Specifically, a highest concentration of 0.2% DMSO or 0.1% ethanol was used in control cells for remdesivir (90 μM) or azithromycin (50 μM), which had no effect on either I_{hERG} or $I_{KCNQ1+KCNE1}$, acutely or chronically. MEM, FBS, G418 (Geneticin), nonessential amino acids, and sodium pyruvate were purchased from Thermo Fisher Scientific (Waltham, MA). Mouse anti-actin primary antibody, EGTA, HEPES, glucose, and electrolytes were purchased from Sigma-Aldrich (St. Louis, MO). Mouse anti-hERG (F-12) primary antibody was purchased from Santa Cruz Biotechnology (Dallas, TX). Horse anti-mouse horseradish peroxidase–conjugated secondary antibody was purchased from Cell Signaling Technology (Danvers, MA). The F-12 primary antibody was used in a 1:1000 dilution, anti-actin primary antibody was used in a 1:2000 dilution, and anti-mouse secondary antibody was used in a 1:20000 dilution.

Statistical Analysis. Data are expressed as means ± S.D. or box plots, including mean, median, and individual data points. A one-way ANOVA with Dunnett's post hoc test or two-tailed Student's *t* test was used to determine statistical significance between the control and test groups. For normalized data, a Wilcoxon matched-pairs test was used to compare currents with each drug to control currents. Statistical analysis was performed with GraphPad Prism 8.4. A *P* value of 0.05 or less was considered statistically significant.

Results

Chloroquine and Hydroxychloroquine, but Not Azithromycin and Remdesivir, Acutely Block hERG Currents. Whole-cell voltage clamp was used to record I_{hERG} in hERG-expressing HEK293 cells. I_{hERG} was elicited using the protocol shown at the top of Fig. 1. The holding potential was −80 mV, which was then depolarized to +50 mV for 4 seconds before repolarizing to −50 mV for 5 seconds. Activation and inactivation of the hERG channel occurs during the depolarizing step, followed by rapid recovery to the open-channel state with slow deactivation during the repolarizing step at −50 mV. This rapid recovery to the open-channel state results in the characteristic tail current of the hERG channel, which is measured to quantify the amplitude of I_{hERG}, as it represents the maximal conductance (Spector et al., 1996). The voltage protocol was delivered every 15 seconds, and I_{hERG} was recorded before (control, black traces) and after drug application to bath solution (treatment, red traces) in the same cells. To compare the effects of the four drugs on I_{hERG}, a 10 μM concentration of each drug was examined. Chloroquine decreased I_{hERG} by more than half, whereas hydroxychloroquine slightly decreased I_{hERG}. However, neither azithromycin nor remdesivir affected I_{hERG} (Fig. 1).

To determine the concentration-dependent effects on I_{hERG}, after recording of control currents, drugs at concentrations ranging from 0.5 to 50 μM were applied consecutively to the same cells during patch-clamp recordings. The peak tail current amplitude at each concentration was normalized to the control current of the cell, 13–19 cells were examined, and summarized data were used to construct the concentration-response relationships. Data were fitted to the Hill equation to obtain the concentration for IC_{50}. Chloroquine blocked I_{hERG}, with an IC_{50} of 3.0 ± 0.3 μM and a Hill coefficient of 0.9 ± 0.1 (n = 6–19 for each concentration). Hydroxychloroquine blocked I_{hERG}, with an IC_{50} of 23.4 ± 7.9 μM and a Hill coefficient of 0.8 ± 0.2 (n = 7–13 for each concentration). Thus, although hydroxychloroquine does block I_{hERG}, it does so with a potency 8-fold less than chloroquine. In addition, neither azithromycin nor remdesivir affected I_{hERG} at 10 or 50 μM (Fig. 2).

To study the effects of chloroquine on the activation-voltage relationships, I_{hERG} was recorded by depolarizing cells from the holding potential of −80 mV to voltages between −70 and 70 mV in 10-mV increments, followed by a repolarizing step to −50 mV. The pulse currents measured at the end of 4-second depolarizing steps and the peak tail currents measured upon the repolarization to −50 mV, normalized to their respective maximal amplitude in control, were plotted against depolarizing (activation) voltages. Chloroquine (10 μM) blocked both pulse and tail currents (Fig. 3). The tail current-voltage relationships were fitted to the Boltzmann equation to obtain the voltage of half-maximal activation ($V_{1/2}$). Chloroquine shifted the $V_{1/2}$ to negative voltages by 6.4 mV (−1.0 ± 2.8 mV in control, −7.4 ± 3.3 mV with chloroquine, $P < 0.05$) without affecting the slope factor (7.5 ± 0.5 in control, 7.2 ± 0.3 with chloroquine, $P > 0.05$, n = 7).

Treatment of hERG-HEK Cells for 24 hours with Remdesivir, but Not Chloroquine, Hydroxychloroquine, or Azithromycin, Increases I_{hERG} and Mature hERG Expression. Drugs may affect hERG function not only by interfering with channel function but also by altering channel

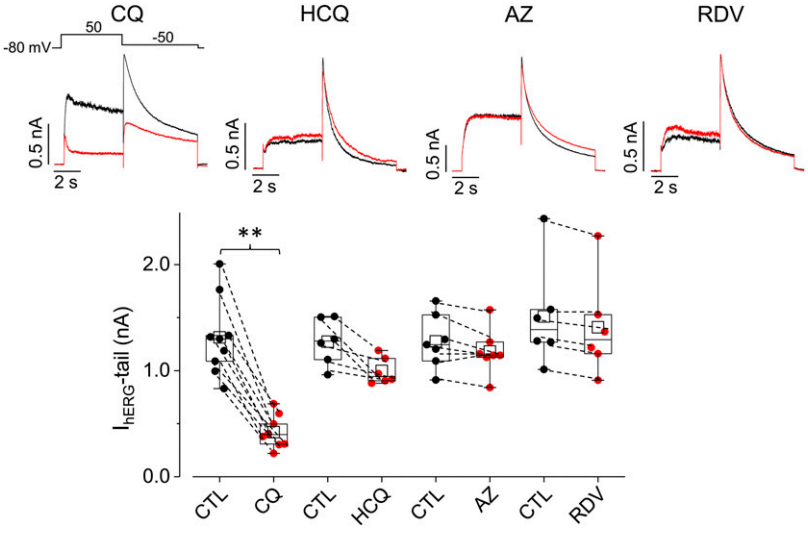

Fig. 1. At a concentration of 10 μM, chloroquine (CQ), but not hydroxychloroquine (HCQ), azithromycin (AZ), or remdesivir (RDV), acutely blocks I_{hERG}. Representative I_{hERG} recorded in control (black) and after drug application (red), with the voltage protocol shown above the current traces. I_{hERG} in the presence of drug as well as control (CTL) is summarized as box plots on the bottom. The square represents the mean, the line within the box represents the median, and original data are shown as dots, with maximal and minimal data indicated with bars. Each dotted line links the current amplitudes recorded before and after drug administration in the same cell. $^{**}P < 0.01$ compared with control.

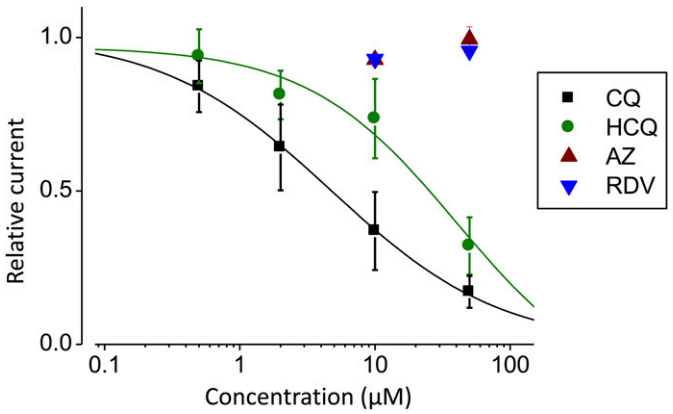

Fig. 2. Concentration-dependent acute effects of chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ), and remdesivir (RDV) on I_{hERG}. I_{hERG} was elicited using the protocol shown in Fig. 1. The peak tail current amplitude at each drug concentration was normalized to the control current. Data from 13–19 cells were summarized and plotted against drug concentrations. The concentration-response relationships were fitted to the Hill equation to obtain the concentration for IC_{50}. Whereas CQ and HCQ blocked I_{hERG} in a concentration-dependent manner, neither AZ nor RDV affected I_{hERG} at 10 or 50 μM.

expression levels (Ficker et al., 2004; Kuryshev et al., 2005; Guo et al., 2007). To determine whether chronic exposure of hERG channels to these drugs affects I_{hERG}, hERG-HEK cells were cultured for 24 hours with 10 μM chloroquine, hydroxychloroquine, azithromycin, or remdesivir, respectively. Once cultured, cells were collected and subjected to whole-cell voltage clamp. Treatment for 24 hours with 10 μM remdesivir increased I_{hERG} by about 2-fold (Fig. 4). When tail currents were plotted against depolarizing voltages and data were fitted to Boltzmann equation, remdesivir increased I_{hERG} without affecting $V_{1/2}$ ($V_{1/2}$: 2.0 ± 1.1, slope factor: 6.6 ± 0.2 in control; $V_{1/2}$: 1.3 ± 2.1, slope factor: 7.3 ± 0.2 with remdesivir, $n = 10$, $P > 0.05$).

To investigate the concentration-dependent effects of chronic treatment with remdesivir on I_{hERG}, hERG-HEK cells were cultured with remdesivir at 0 (control), 1, 3, 10, 30, and 90 μM for 24 hours. However, a clear sigmoidal concentration-dependent effect was not observed (Fig. 5A). Although an increase in I_{hERG} was observed at 10 μM, 30 or 90 μM did not cause additional increases but instead decreased I_{hERG} slightly compared with 10 μM. Chronic treatments with drugs may lead to a changed hERG protein expression (Kuryshev et al., 2005; Guo et al., 2007; Lamothe and Zhang, 2013; Wang et al., 2014; Sutherland-Deveen et al., 2019). To investigate whether an altered hERG protein expression is associated with the changes in I_{hERG}, we performed Western blot analysis. As shown in Fig. 5B, after a 24-hour culture at 10 μM, chloroquine, hydroxychloroquine, and azithromycin had no effect on hERG expression. However, remdesivir increased the 155-kDa hERG band density, which may underlie the I_{hERG} increase. Subsequently, hERG-HEK cells were treated with varying concentrations of remdesivir for 24 hours and subjected to Western blot analysis to determine whether the increase of mature hERG expression was concentration-dependent. As shown in Fig. 5C, remdesivir increased 155-kDa hERG expression in a concentration-dependent manner, although the increase at the highest concentration (90 μM) appeared less prominent than that of 30 μM.

Chloroquine, Hydroxychloroquine, Azithromycin, and Remdesivir Do Not Affect $I_{KCNQ1+KCNE1}$. In addition to I_{Kr}/hERG, I_{Ks} (which is encoded by KCNQ1 and KCNE1) is another important current for cardiac repolarization (Sanguinetti et al., 1996; Barhanin et al., 1996). We recorded KCNQ1 + KCNE1 current ($I_{KCNQ1+KCNE1}$) from HEK cells that stably express both KCNQ1 and KCNE1. After recording control currents, drugs were added to the bath solution, and currents were recorded again in the same cells. For analysis, currents in the presence of drugs are normalized to their respective controls and presented as relative current for each drug. Chloroquine, hydroxychloroquine, azithromycin, or remdesivir did not acutely affect $I_{KCNQ1+KCNE1}$ (Fig. 6). To investigate whether chronic treatments with these drugs affect $I_{KCNQ1+KCNE1}$, KCNQ1+KCNE1-HEK cells were cultured in control or with 10 μM of chloroquine, hydroxychloroquine, azithromycin, or remdesivir for 24 hours and then examined with whole-cell patch-clamp recordings using the voltage protocol shown in Fig. 4A. Current amplitudes upon the 50-mV depolarizing step in each group were demonstrated in box plots. None of the four drugs chronically affected $I_{KCNQ1+KCNE1}$ (Fig. 7).

Discussion

In the present study, we investigated the acute and chronic effects of chloroquine, hydroxychloroquine, azithromycin, and remdesivir on two primary repolarizing K^+ currents, I_{hERG} and $I_{KCNQ1+KCNE1}$. Our data showed that chloroquine blocked I_{hERG}, with an IC_{50} of 3.0 μM, whereas hydroxychloroquine blocked I_{hERG} with a potency 8-fold less, with an IC_{50} of 23.4 μM. Neither azithromycin nor remdesivir blocked I_{hERG} at concentrations up to 50 μM. When drugs were added to the cell culture medium for 24 hours, chloroquine, hydroxychloroquine, and azithromycin did not affect I_{hERG} and hERG

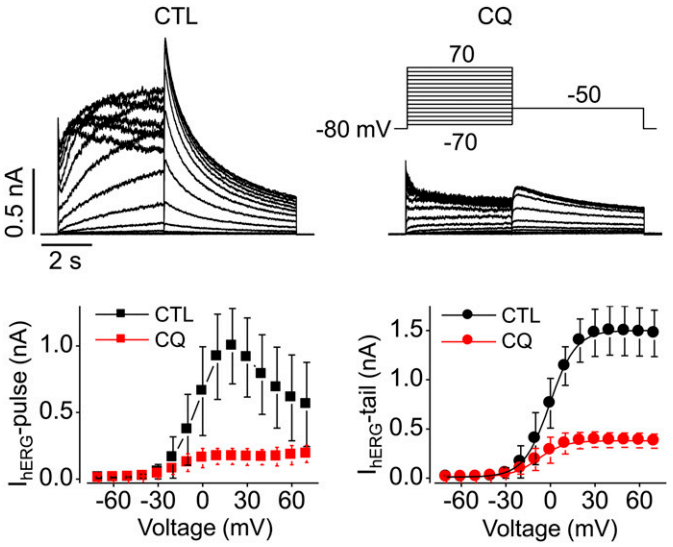

Fig. 3. Acute effects of chloroquine (CQ) on activation-voltage relationships of hERG channels. Family of I_{hERG} was recorded by the voltage protocol shown above the current traces. The pulse currents measured at the end of 4-second depolarizing steps and the tail currents measured upon the repolarization to −50 mV were plotted against depolarizing (activation) voltages. The tail current-voltage relationships were fitted to the Boltzmann function to obtain the $V_{1/2}$ and slope factors.

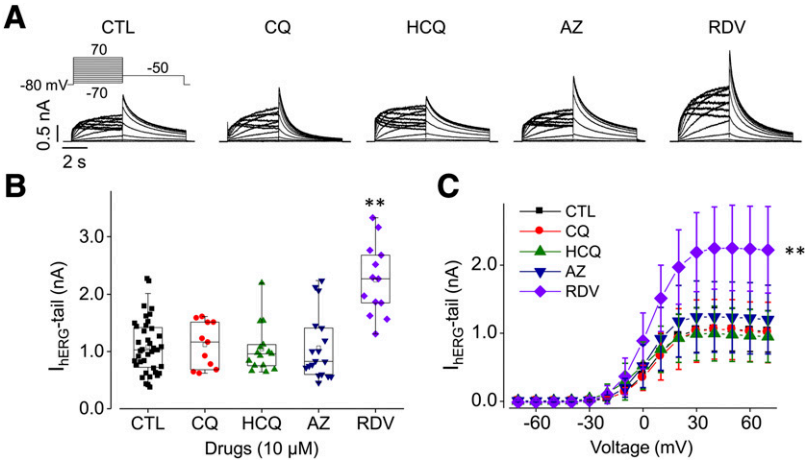

Fig. 4. Chronic effects of chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ), and remdesivir (RDV) on I_{hERG}. (A) Representative hERG currents in CTL cells and those cultured with 10 μM CQ, HCQ, AZ, or RDV for 24 hours. (B) Box plots showing maximal tail currents upon repolarization to −50 mV from hERG-HEK cells cultured in control or with 10 μM CQ, HCQ, AZ, or RDV for 24 hours. $^{**}P < 0.01$ compared with control. (C) Tail current-voltage relationships fitted to the Boltzmann equation function of I_{hERG} recorded from cells cultured in control or with 10 μM CQ, HCQ, AZ, or RDV for 24 hours. $^{**}P < 0.01$ compared with control at voltages >0 mV.

expression levels. However, remdesivir at 10 μM chronically increased I_{hERG} by 2-fold, which was associated with an increased expression of the mature hERG proteins. Furthermore, none of these drugs acutely or chronically affected $I_{KCNQ1+KCNE1}$. These findings are summarized in Fig. 8.

Since reliable information on drug concentrations or doses for treating COVID-19 is lacking, doses that proved effective and safe in the treatment of other diseases, such as malaria, can be considered. For chloroquine, peak serum concentrations of 0.82 μM (263 ng/ml) and 1.37 μM for malaria treatment (Walker et al., 1983; Rombo et al., 1987) were reported, and a target plasma concentration not above 2.5 or 3.0 μM has been proposed (Smit et al., 2020; White et al., 2020). For hydroxychloroquine, the average serum/plasma hydroxychloroquine concentrations for patients with systemic lupus erythematosus were found below 480 ng/ml (1.43 μM), which is 2-fold of the EC_{50} of 0.72 μM obtained in an in vitro study targeting SARS-CoV-2 with hydroxychloroquine for 48 hours (Carlsson et al., 2020; Balevic et al., 2020; Yao et al., 2020). For azithromycin, the plasma/serum concentration was 240–650 ng/ml (0.32–0.87 μM) during the treatment of lung infection (Jeong et al., 2016) and was 565.53 ng/ml (0.75 μM) in a clinical study involving healthy volunteers (Matzneller et al., 2013). For remdesivir, the mean plasma concentration-time profiles after intravenous administration were documented in two critically ill patients with COVID-19 who recovered (Tempestilli et al., 2020). In these two patients, 200 mg remdesivir was intravenously administered over 1 hour on day 1, followed by one-time 100-mg administration daily for an additional 12 days. On days 3–9, blood samples were collected immediately after, at 1 and 24 hours after remdesivir administration. It was shown that immediately after remdesivir administration, mean peak serum concentrations for the two patients were 2737 and 3317 ng/ml (4.54 and 5.50 μM). Plasma concentration of remdesivir decayed rapidly; 1 hour after infusion, they were 80.7 and 171 ng/ml (0.13 and 0.28 μM), respectively; and 24 hours after infusion, they were below the limit of quantification (Tempestilli et al., 2020). It is important to note that drugs may bind to serum proteins, and their free concentrations remain unknown but are expected to be even lower. Thus, it appears that all these drugs can reach single-digit micromolar levels in clinical

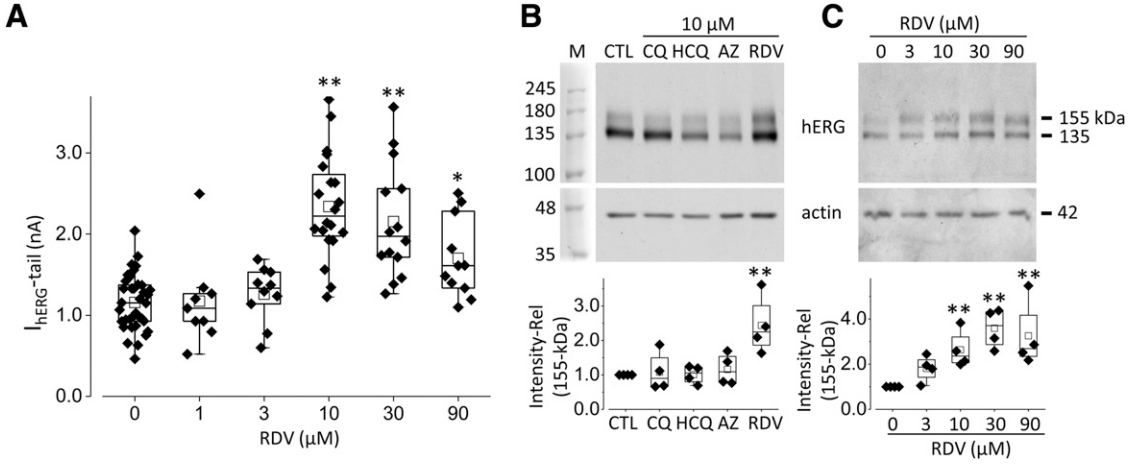

Fig. 5. Concentration-dependent chronic effects of remdesivir (RDV) on I_{hERG} and hERG expression. (A) Box plots showing maximal tail currents upon repolarization to −50 mV from hERG-HEK cells cultured in control or with various concentrations of RDV for 24 hours. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with absence of drug. (B) Western blot showing hERG expression of hERG-HEK cells cultured in CTL or with 10 μM chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ), or RDV for 24 hours. $n = 4$, $^{**}P < 0.01$ compared with control. (C) Western blot showing hERG expression of hERG-HEK cells cultured in the absence or presence of various concentrations of RDV for 24 hours. $n = 4$, $^{**}P < 0.01$ compared with absence of drug.

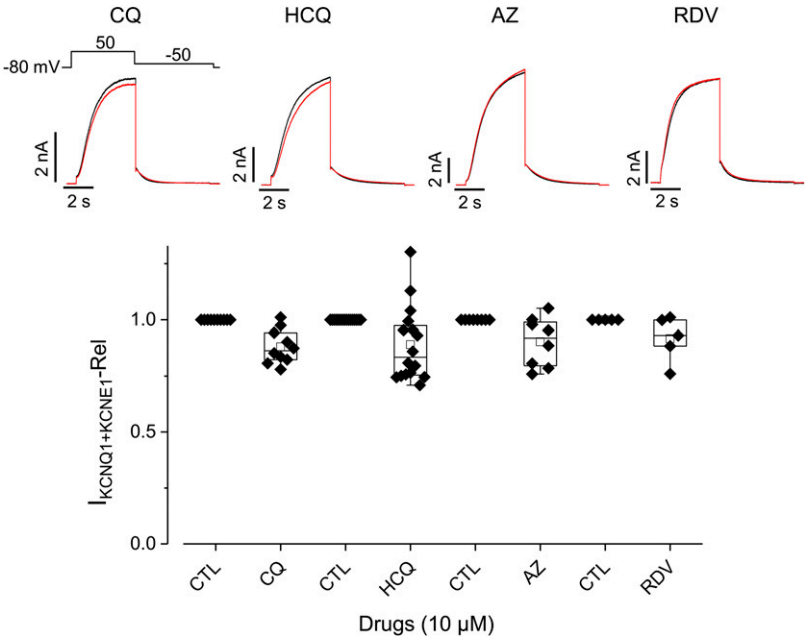

Fig. 6. Chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ), or remdesivir (RDV) does not acutely affect $I_{KCNQ1+KCNE1}$. Top: representative $I_{KCNQ1+KCNE1}$ traces recorded with the shown voltage protocol before and after 10 μM CQ, HCQ, AZ, or RDV. Bottom: peak current amplitudes in the presence of drug were normalized to the respective CTL (in the absence of drug) in the same cell and plotted as relative (Rel) $I_{KCNQ1+KCNE1}$.

settings. While 10 μM of each of the four drugs was examined during whole-cell voltage-clamp recordings, only chloroquine significantly decreased I_{hERG} (Fig. 1). Chloroquine-induced hERG block was previously reported (Traebert et al., 2004; Sánchez-Chapula et al., 2002).

Our results showed that chloroquine and hydroxychloroquine acutely blocked I_{hERG} in a concentration-dependent manner, with hydroxychloroquine having 8-fold less potency than chloroquine (IC_{50} being 23.4 vs. 3.0 μM). The ~8-fold difference in hERG-blocking potency is due to the hydroxyl group, which is the only difference between hydroxychloroquine and chloroquine. Molecular mechanisms underlying a hydroxyl addition–induced reduction of hERG block by chloroquine warrant further investigation. As an adverse unwanted effect, drug-induced hERG block and potential LQTS represent a significant concern for drug safety (Finlayson et al., 2004; Shah, 2005; Kannankeril et al., 2010). The addition of a hydroxyl to the ethyl group of chloroquine to form hydroxychloroquine, which retains drug properties while reducing hERG-blocking potency, would be of great interest for drug development.

We also investigated the chronic effects of the four drugs on the current and expression of hERG channels and found that none of them chronically decreased I_{hERG}. However, treatment of cells with 10 μM remdesivir for 24 hours increased I_{hERG} by 2-fold, and an increased channel expression is responsible for increased I_{hERG} (Figs. 4 and 5). The molecular mechanisms underlying the remdesivir-induced increase in I_{hERG} and hERG protein expression are currently unknown and warrant future investigation. The clinical relevance of remdesivir-mediated increase in I_{hERG} is also unknown. Some hERG mutations can cause short QT syndrome and arrhythmias due to the increased I_{hERG} (Brugada et al., 2004; Schimpf et al., 2005). The possibility of drug-induced QT shortening has also been previously recognized

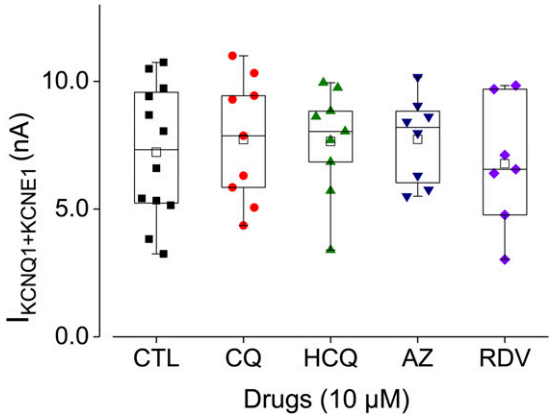

Fig. 7. Chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ), or remdesivir (RDV) does not chronically affect $I_{KCNQ1+KCNE1}$. $I_{KCNQ1+KCNE1}$ was recorded using the voltage protocol shown on the top left of Fig. 4A. $I_{KCNQ1+KCNE1}$ was measured at the end of the 4-second depolarizing step to 50 mV from KCNQ1+KCNE1-HEK cells cultured in control or with 10 μM CQ, HCQ, AZ, or RDV for 24 hours. Data are shown in box plots for various treatments.

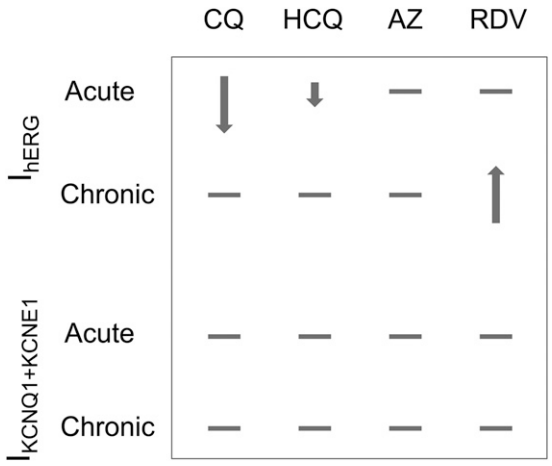

Fig. 8. Summary of the acute and chronic effects of chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ), or remdesivir (RDV) on I_{hERG} and $I_{KCNQ1+KCNE1}$. Upper arrow: increase; down arrow: decrease; horizontal line: no effect.

(Schimpf et al., 2012; Huang et al., 2019). However, drug-induced short QT syndrome is rare. Remdesivir chronically increased I_{hERG} at concentrations that may be above clinical concentrations (Tempestilli et al., 2020). On the other hand, whether remdesivir can counteract against chloroquine- or hydroxychloroquine-induced I_{hERG} inhibition remains to be an important question that may warrant future basic and clinical investigations.

In addition to I_{Kr} encoded by hERG, I_{Ks} encoded by KCNQ plus KCNE1 is the other important potassium current responsible for cardiac repolarization. We also examined the effects of chloroquine, hydroxychloroquine, azithromycin, and remdesivir on KCNQ + KCNE1 channels. Our data showed that none of the four drugs affect $I_{KCNQ1+KCNE1}$ acutely or chronically (Figs. 6 and7).

Overall, out of the COVID-19 drugs examined in our study, at clinically relevant levels, chloroquine acutely caused a significant decrease in I_{hERG}. Although hydroxychloroquine also blocks hERG, it is 8-fold less potent than chloroquine, and block may only occur at concentrations above those observed in patient serum. These data are in line with a recent study showing that hydroxychloroquine is safe for COVID-19 and not associated with a risk of ventricular arrhythmia due to drug-induced QTc prolongation (Sogut et al., 2021). Our data also revealed that I_{hERG} was chronically increased by remdesivir at concentrations that may be beyond clinically relevant levels.

It remains to be determined whether QT-prolonging drugs are more likely to induce torsades de pointes arrhythmia in patients with COVID-19. Hospitalized patients with COVID-19 may suffer from hypoxia, fever, electrolyte abnormalities (especially hypokalemia), and viral- or autoimmune-induced myocardial injury (Hu et al., 2021; Zeng et al., 2020; Clerkin et al., 2020). We have previously demonstrated that hypoxia, fever, hypokalemia, and protease activation can damage or facilitate the damage of hERG function (Guo et al., 2009; Lamothe et al., 2016; Zhao et al., 2016; Lamothe et al., 2017). In fact, hospitalized patients with COVID-19 display respiratory symptoms such as shortness of breath, and they also display cardiac arrhythmias (Wang et al., 2020; Du et al., 2020). In addition, patients with COVID-19 receiving chloroquine or hydroxychloroquine as a therapy may respond to other medications differently because chloroquine and hydroxychloroquine are known to inhibit cytochrome P450 enzymes (Rendic and Guengerich, 2020). Cytochromes P450 are responsible for the metabolism of most foreign substances, including 70%–80% of clinically used drugs (Zanger and Schwab, 2013), and coadministration of cytochrome P450–inhibiting drugs may increase plasma levels of other drugs that inhibit I_{hERG}, enhancing the risk of drug-induced LQTS (Furst et al., 2002). Besides K^+ current inhibition, drugs may be proarrhythmic through other mechanisms. For example, although it was reported that clinical levels of azithromycin neither blocks I_{hERG} in hERG-HEK cells nor prolongs QTc in anesthetized dogs (Thomsen et al., 2006), another study reported that acute treatment of azithromycin results in a concentration-dependent block of Na^+ currents, whereas chronic azithromycin treatment increases sodium current through SCN5A (Sodium Voltage-Gated Channel Alpha Subunit 5) by 2-fold (Yang et al., 2017). Finally, patients with COVID-19 with pre-existing inherited arrhythmia syndromes may be more likely to develop LQTS (Wu et al., 2020). Because of these reasons, attention should be paid to monitoring ECG recordings for patients with COVID-19 to prevent and treat arrhythmias in a timely manner.

Authorship Contributions:

Participated in research design: Zhang.
Conducted experiments: Szendrey, Guo, Li, Yang.
Performed data analysis: Szendrey, Guo, Li, Yang.
Wrote or contributed to the writing of the manuscript: Szendrey, Zhang.

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