Potential therapeutic drugs for COVID-19 risk prolonging QT interval targeting hERG channel

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Abstract

The COVID-19 caused by SARS-CoV-2 poses a huge challenge to the medical system, especially the safe and effective COVID-19 treatment methods, forcing people to look for drugs that may have therapeutic effects as soon as possible. Some old drugs have shown clinical benefits after a few small clinical trials attracting great attention. Clinically, however, many drugs including those currently shown to be effective against COVID-19 such as chloroquine, hydroxychloroquine, azithromycin and lopinavir/ritonavir may cause cardiotoxicity through acting on cardiac potassium channel, hERG channel due to their off-target effect. Blocking of hERG prolongs QT intervals on the electrocardiogram and thus might induce severe ventricular arrhythmias and even sudden cardiac death. Therefore, while focusing on the efficacy of COVID-19 drugs, the fact that they block hERG to cause arrhythmias can not be ignored. To develop safer and effective drugs, it is necessary to understand the interactions between drugs and hERG channels and the molecular mechanism behind this high affinity. In this review, we focus on the biochemical and molecular mechanistic aspects of related drug blockade in the hERG trying to provide insights into the QT interval prolongation caused by potential therapeutic drugs for COVID-19 and hope to weigh the risks and benefits when using related drugs.

Keywords: COVID-19; drugs; hERG channel; QT interval; long QT syndrome (LQTS)

Introduction

By the end of May 2020, in the six months since the end of 2019, the COVID-19 caused by SARS-CoV-2 swept the world, with more than 6 million infections and over 350,000 dead, making it the most terrifying killer of human life recently. The COVID-19 pandemic urgently requires effective and safe treatments that enable people to strive for rapid research and application of drugs, and at least 12 potential COVID-19 treatments are now being tested. This is a race against COVID-19(Kupferschmidt and Cohen 2020). However, although a series of drugs approved for other indications and a variety of research drugs are being studied in hundreds of clinical trials (*available at ClinicalTrials.gov*) around the world, there is currently no FDA approval of COVID-19 drugs(Jan *et al.* 2020, Panel. 2020). In the treatment guidelines issued on April 21, the National Institutes of Health (NIH) pointed out that at present, there are no drugs proven to be safe and effective in the treatment of COVID-19(Panel. 2020).

Early, some compounds have shown to be beneficial to patients after small clinical randomized trials, such as antimalarial drugs(Mercuro*et al.* 2020, Saleh *et al.* 2020, Wang *et al.* 2020, Yao *et al.* 2020), antiviral drugs(Cao *et al.* 2020a, Hung*et al.* 2020), and so on. However, the evidence of the effective treatment of these drugs is still insufficient, and part of them, such as chloroquine, due to the advocate of some public

figures, may hinder the research of new potential therapeutic drugs(Ledford 2020). In addition, a noticeable problem is that these drugs may increase the risk of QT interval prolongation and ventricular arrhythmias(Giudicessi*et al.* 2020, Naksuk *et al.* 2020). A statement from the Canadian Cardiovascular Society (CCS) recommended that unnecessary drugs be discontinued, especially chloroquine, hydroxychloroquine, azithromycin, and lopinavir/ritonavir, and baseline electrocardiograms should be performed in high-risk patients(Sapp *et al.* 2020). In such cases, we have reviewed the QT prolongation reports of therapeutic drugs of COVID-19 and found that the use of drugs mentioned above alone or in combination has the risk of prolonging the QT interval, and even chloroquine and hydroxychloroquine have been listed as drugs that can cause direct myocardial toxicity. More importantly, patients with potential congenital arrhythmias, particularly long QT syndrome (LQTS), are at greater risk of fatal arrhythmias, such as Torsades de Pointes (TdP), during receiving the above drugs(Giudicessi *et al.* 2020, Kannankeril *et al.* 2010).

It is well known that many factors participate in prolonging QT interval, mainly divided into congenital LQTS (cLQTS) caused by genetic mutation and acquired LQTS (aLQTS) mainly due to the drug off-target effects, named as drug-induced LQTS (diLQTS), which can both cause TdP(Cubeddu 2016, Schwartz and Woosley 2016). Importantly, the hERG (Kv11.1) channel is the main target of drugs in diLQTS(Kannankeril*et al.* 2010, van Noord *et al.* 2010). Many drugs have been withdrawn from the clinic because of their severe potential arrhythmia targeting the hERG channel, including antibiotics, antivirals, antifungals, antimalarials, antidepressants, and so on(Cubeddu 2016, Mladěnka *et al.* 2018). The potential for hERG involvement in drug-associated arrhythmia is so strong that we need to pay more attention to the biological characteristics of hERG itself(Butler*et al.* 2019). Explaining and understanding the high affinity of drugs with the hERG channel requires revealing the molecular basis for hERG interactions with drugs to produce safer treatment drugs for diseases including COVID-19.

1. hERG channel
2. **The significance of the hERG channel**

hERG channel is also known as Kv11.1 channel or ERG1 potassium channel. hERG gene encodes the pore-forming alpha subunit of a fast component of the delayed rectifier potassium channel (I_{Kr}), which plays a fundamental role in the three-phase repolarization of the ventricular action potential, i.e., the repolarization of cardiac myocytes(Vandenberg *et al.* 2012). The dysfunction of hERG channel contributes to partial or complete reduction of I_{Kr}current, resulting in prolonged action potential duration(Smith *et al.* 2016), manifested as a prolonged period of QT interval on electrocardiogram (ECG) (Figure1b), and if the extension exceeds the normal range (440 ms in men, 460 ms in women) becomes LQTS(Schwartz and Ackerman 2013, van Noord *et al.* 2010). Clinically, LQTS caused by hERG deficiency is called type 2 LQTS (LQT2), which is the second most common subtype of LQTS(Kapplinger *et al.* 2009).

The electrical activity of the heart is mediated by regulating the channels in which ions flow into and out of cardiomyocytes. Theoretically, the ion currents that constitute the ventricular action potential include inward and outward currents, and the balance of outward and inward current is the key to the formation of normal action potential duration. Na^+ and L-type Ca^{2+} currents (I_{Na} and I_{CaL}) are the most important inward currents in cardiomyocytes, and k^+ current (I_K) is the major outward currents. Increased inward current or weakening of outward currents can result in a prolongation of action potential duration, leading to a prolonged QT interval (Figure1a)(van Noord*et al.* 2010). Excessive prolongation can facilitate the production of early after depolarization (EAD) in three-phase repolarization that will trigger TdP and even ventricular fibrillation if it reaches its threshold(Albert and Schuller 2014).

A decrease in outward potassium currents causing longer repolarization time due to the blocking of hERG, the effect of which is consistent with strengthening sodium or calcium currents. Therefore, the effect of drug inhibition on hERG channels appears to be corrected by drugs that block I_{Na} and I_{CaL}. A classical example is amiodarone, a widely used class III antiarrhythmic drug, that shows an inhibition of multiple ion channels to balance the blocking of I_{Kr}, so it has rare malignant arrhythmia events generated by potassium channel blockage. However, cardiac and non-cardiac QT-prolonging drugs are often easy to target the hERG channel but not other ion channels to cause arrhythmias. Drugs with single ion channel effects and non-cardiac drugs

that act on hERG are inevitably potentially arrhythmogenic. Given the importance of this matter, many studies have focused on revealing hERG biological characteristics to explain possible mechanisms of drug action and made some progress.

hERG biogenesis and quality control

hERG (ERG1) gene or *KCNH2* is located on chromosome 7q35-36(He*et al.* 2020, Vandenberg *et al.* 2012). There are at least three alternative *KCNH2* transcription start site transcribed in the nucleus to produce mRNA, which encodes transcripts called KCNH2-1a, KCNH2-1b, and KCNH2-3.1, respectively. Nonsense-mediated mRNA decay (NMD) maintains KCNH2 mRNA stability and protects cells from abnormal protein production through degradation of nonsense mutation possessing premature termination codons(Gong *et al.* 2007). Detected by NMD, qualified mRNA migrates to the ribosome for translation to generate the nascent polypeptide chain, and then undergoes core glycosylation (135KDa) in the endoplasmic reticulum (ER). The correctly folded peptide chain will enter the Golgi apparatus for further complex glycosylation (155KDa) (Figure2)(Foo *et al.* 2016, He *et al.* 2020, Nogawa and Kawai 2014).

The process of hERG protein biogenesis synthesis including folding, assembly, and translocation requires assistance from molecular chaperones, the most important of which is the heat shock protein (Hsp) family(Ficker *et al.* 2003, Iwai *et al.* 2013, Li *et al.* 2011, Zhang *et al.* 2014). Hsp70 and its homologous Hsc70 participate in the folding of nascent hERG and is believed that to modulate the hERG channel in a reciprocal way(Li *et al.* 2011, Zhang *et al.* 2014). Hsp70 suppresses hERG ubiquitination and increases hERG levels while Hsc70 reverses these effects (Li *et al.* 2011). Hsp90 reduces aggregation of misfolded protein and hERG ubiquitination by preventing interaction between endogenous C-terminus of Hsp70-interacting protein (CHIP) and hERG proteins(Ficker *et al.* 2003, Iwai *et al.* 2013). Specifically, Hsp40, as a co-chaperone for Hsp70, is thought to regulate hERG degradation(Walker*et al.* 2010). Type I Hsp40s, DNAJA1, and DNAJA2 can weaken the interaction between hERG and Hsc70 to promote the early degradation of hERG(Walker *et al.* 2010). Moreover, DNAJB12 and DNAJB14 also promote the tetramer assembly of hERG channel subunits through a mechanism independent of Hsp70 in the ER (Figure2)(Li *et al.*2017).

The mature protein will be transported to the plasma membrane to function. In reverse, if protein glycosylation cannot be done properly, the unqualified protein will retain in the ER or return to the ER from Golgi, and then up-regulate the levels of the ER molecular chaperone to cope with the condition. Nevertheless, if losing balances it will trigger ER stress coping responses, namely unfolded protein response (UPR) associated with the ubiquitin-proteasome system (UPS) for degradation, named ER-associated degradation (ERAD) (Figure2)(Wang*et al.* 2015). Another way of quality control is to rely on ubiquitination at the plasma membrane (PM) essential for cell homeostasis(Apaja and Lukacs 2014, Foo *et al.* 2016, Zhang*et al.* 2014). hERG protein on PM will internalize into clathrin-coated vesicles upon ubiquitination under the assistance of Caveolin (Cav), dynamin, and Arf6 (Zhang *et al.* 2014). Internalized proteins labeled by ubiquitin can be degraded by lysosomes or proteasomes, otherwise recycled back to the PM (Figure3)(Foo *et al.* 2016, Zhang *et al.* 2014). Many factors such as temperature, hypoxia, pH, potassium concentration, sex hormones, etc. can affect hERG protein maturation. Therefore, by altering the relevant factors that affect hERG maturation in the body, the drug can produce an effect of inhibiting or enhancing the function of hERG, which may further promote it to be the target of many drugs and also makes the regulation of hERG channel generation very complicated.

hERG structure and gating kinetics

hERG channel shares structural homology with other Kv channels(Whicher and MacKinnon 2016). The hERG channel act as a tetramer and four hERG subunits are arranged in a ring-shaped manner rather linearly arranged on the membrane(Vandenberg *et al.* 2012). Each subunit has six transmembrane fragments (S1 -S6), the N-terminal Per-Arnt-Sim (PAS) domain, and the C-terminal cyclic nucleotide-binding domain (cNBD) (Barros *et al.* 2020, Shi *et al.* 2020, Warmke and Ganetzky 1994). S1-S4 functions as a voltage-sensing domain (VSD) and S4 provides the most important component of transmembrane voltage sensing with several positively charged residues(Barros *et al.* 2012). Two other transmembrane helices S5-S6 coupling

with the intermediate pore loops of the four subunits constitutes the ion permeation pore-gate domain (PD) consisting of the permeation pathway, the potassium selectivity filter, and the channel gate (Figure4b)(Barros *et al.*2020, Wang *et al.* 2011). Importantly, N-terminal and C-terminal are respectively involved in the inactivation of the hERG channel (N-type inactivation and C-type inactivation)(Barros *et al.* 2012, Shi *et al.* 2020) and are also the binding sites of Hsp(Iwai*et al.* 2013). Therefore, an interaction between hERG blocking drugs and Hsp may involve a change of gating kinetics.

Recently, the new hERG structure obtained from cryo-electron microscopy (cryo-EM) has aroused great attention, deleted most of the expected unstructured cytoplasmic regions (Δ141–350 at the N-terminal and Δ871–1005 at the C-terminal) (Figure4a)(Wang and MacKinnon 2017). The structure with a resolution of up to 3.8 Å retains the gating properties similar to full-length channels because it does not change the major function of the hERG channel already recognized. It provides new insights into the molecular basis of high-affinity for hERG drugs block, the binding of hERG activators, and the molecular basis of hERG unique gating kinetics by demonstrating the important special structure of compounds binding(Butler *et al.* 2019, Vandenberg *et al.*2017).

Similar to other voltage-gated potassium channels, the hERG channel has three different conformational states, namely closed, open, and inactivated, respectively(Shi *et al.* 2020, Vandenberg *et al.* 2017, Vandenberg *et al.* 2012). However, hERG has its unique gating kinetics with slow activation and deactivation, but faster inactivation and recovery from inactivation, and these state changes depend on voltage changes(Shi *et al.* 2020, Vandenberg *et al.* 2017), leading to the inward rectifying characteristic(Smith*et al.* 1996, Spector *et al.* 1996). The altering rate of inactivation and deactivation reflects the potential potency of drug block.

In the early stage of the action potential, the hERG channel slowly opens, but rapidly inactivates. As depolarization voltage continues to decrease, hERG gradually recovers from inactivation(Witchel *et al.* 2001). Due to recovery from inactivation, hERG currents improve during repolarization followed by a much slower deactivation (Figure1c)(Barros *et al.* 2012, Vandenberg *et al.* 2017). Drugs can affect the opening and closing process of the channel, resulting in the activation curve shifts left or right and the tail current decay process becomes faster or slower. More recently, the selectivity filter at the hERG channel was reported as a pharmacological master mechanism to open potassium channels(Schewe *et al.* 2019). That offers a substantial boost in designing channel activators to rescue aLQTS or diLQTS. Because of the unique structure and gating kinetics of the hERG channel, in-depth understanding and research of molecular basis under different channel states will bring us help to develop safer drugs.

Pharmacology of hERG channel

As we all know, I_{Kr} is the target of class III antiarrhythmic drugs like dofetilide, amiodarone, D-sotalol, etc. These drugs produce voltage- and dose-dependent hERG channel blockade. DiLQT results from off-target inhibition by a variety of compounds, most commonly because the drugs directly block the ion pathways(van Noord*et al.* 2010, Wang and MacKinnon 2017). In the past decades, considerable progress has been made in understanding the structural characteristics of drugs combined with hERG channels. The following insights were gradually formed: (i) Blockers gain access to a binding site that require the channel opening due to the drug binding located in the central cavity of the channel PD(Mitcheson *et al.* 2000, Vandenberg *et al.* 2012). (ii) Two key aromatic residues (Tyr652 And Phe656) on S6 are the most critical drug-binding sites(Butler*et al.* 2019) and blockers can be trapped in the inner vestibule by closing the activation gate once them inside the pore (Figure4c, Figure1d)(Tamargo *et al.* 2004). (iii) The cavity of the hERG channel is so large (the lack of Pro-X-Pro sequences in the S6) that it is sufficient to capture drugs at least as large as MK-499(Mitcheson*et al.* 2000, Tamargo *et al.* 2004). (iv) Most high-affinity drugs preferentially combine the inactivated state rather than the open state because mutations losing of inactivation reduce the affinity of some blockers, while mutations accelerating inactivation enhance the blocking potency(Perrin *et al.* 2008, Vandenberg *et al.*2017).

3.1 Structural basis of drug binding

Many studies have reported the importance of multiple aromatic residues for drug blockade based on the ho-

mology difference between other Kv channels via the mutagenesis of individual residues in the S6 helix(Han *et al.* 2015, Han *et al.* 2011, Jie *et al.* 2017, Rajamani *et al.* 2006, Sănchez-Chapula *et al.* 2003, Sánchez-Chapula *et al.* 2002, Takemasa *et al.* 2008). Among them, two aromatic residues of S6 Tyr652 and Phe656 is particularly significant. Their mutations can attenuate the drug blocking effect, confirming their high affinity for drug binding. It is believed that the Y652A and F656A mutations decrease the inhibitory effect by 17 times and 75 times, respectively(Helliwell *et al.* 2018). Chloroquine generates the effect of channel block can be restored by mutation of Tyr-652 showing that these key sites to block channels(Sánchez-Chapula *et al.* 2002).

In addition to the two key residues mentioned above, other residues at the bottom of the pore helix, Thr623, Ser624, and Val625 and Phe557 on the S5 helix also contribute to drug binding, equally potent as Y652(Mitcheson *et al.* 2000, Saxena *et al.* 2016). The cryo-EM structure does not alter the basic feature of the hERG channel and retains all the functions essential for drug binding, which illustrates why these special amino acids of drug biding are so important(Wang and MacKinnon 2017). The positions of these amino acids in the sequence and molecular structure are highlighted and they are arranged on the surface of elongated, relatively hydrophobic pouches protruding from the central cavity(Wang and MacKinnon 2017). These pockets provide potential interaction sites for hERG blockers(Butler *et al.* 2019). To elucidate the chemical basis of drug binding, higher resolution structures will be needed and in combination with molecular dynamics simulations, it can accurately identify where and how drugs bind to the hERG channel, and why they bind differently to distinct conformation states of the channels.

Disruption of hERG channel Trafficking

As indicated earlier, the drugs binding into the pore region of the hERG channel exerts a direct blocking effect that disrupts the conduction of ions through the pore. Many different structurally drug groups cause QT prolongation targeting hERG through disrupting the trafficking of protein(Dennis *et al.* 2007, Kuryshev *et al.* 2005, Nogawa and Kawai 2014). These drugs include arsenic(Ficker *et al.* 2004), pentamidine(Kuryshev *et al.* 2005), fluconazole(Han *et al.*2011) and ketoconazole(Takemasa *et al.* 2008), fluoxetine(Hancox and Mitcheson 2006), cardiac glycosides(Wang *et al.* 2007), rosuvastatin(Feng *et al.* 2019), thioridazine(Liu *et al.*2020) and so on. Arsenic trioxide (As_2O_3) is the first example of a drug that produces hERG liability by inhibition of channel protein trafficking through disrupting hERG-chaperone complexes(Ficker *et al.* 2004). Up to 40% of all hERG blockers exert combined hERG block and trafficking inhibition(Dennis *et al.* 2011). And as increasing drugs target the hERG channel, more drug studies have confirmed that the above two mechanisms can work together and probably are mediated by different mechanisms (Han *et al.* 2011, Hancox and Mitcheson 2006, Rajamani *et al.* 2006, Takemasa *et al.* 2008). It made the interfering effect of the hERG channel more complicated.

In general, numerous drugs reduce the generation of hERG mature 155-kDa form by disrupting the forward trafficking of hERG protein from ER to Golgi. This process is associated with molecular chaperones that help protein folding and assembling. For instance, inhibition of chaperone Hsp90 prevents maturation and promotes the proteasome degradation of hERG, thereby reducing the number of mature channels that can be integrated into the cell membrane(Cubeddu 2016). Different maturation of hERG can be estimated by comparing the expression levels of the two forms of this protein.

The mechanism of interference with hERG transport by drugs is similar to the class II mutation of LQT2 occurring due to congenital hERG mutation like A561V missense mutation, causing hERG channel trafficking defects(Delisle *et al.* 2004, Smith *et al.* 2016). For diLQTS and cLQTS, protein retention in the ER will induce high expression of molecular chaperones to promote protein maturation. Particularly, probucol reduces hERG expression from the cell membrane via accelerating Cav1 turnover(Figure3)(Guo *et al.* 2011, Guo*et al.* 2007). The antidepressant desipramine not only inhibits the forward transport of hERG but also increases channel endocytosis and ubiquitination degradation (Figure3)(Dennis *et al.* 2011). Notably, in the conditions of low extracellular potassium concentration, the caveolin-dependent internalization of hERG is also enhanced (Massaeli *et al.* 2010), which is attributed to the monoubiquitination of hERG at the membrane(Sun *et al.* 2011). There is currently no relevant evidence to support COVID-19 drugs that interfere with the maturation of hERG. The safe application of these drugs may require detailed information about

hERG blocking intracellular mechanisms.

Other mechanisms

Another issue worth considering is that many drugs may alter drug metabolism which may further increase plasma levels of drugs that extend QT interval. Pharmacokinetic interactions usually involve agents metabolized by cytochrome P450 enzymes(van Noord *et al.* 2010). P450 (CYP) 3A is the highest expression subfamily, including isoforms CYP3A4, CYP3A5, CYP3A7, and CYP3A43(Eichelbaum and Burk 2001). CYP3A4 is the isoforms expressed in the liver and intestine(Eichelbaum and Burk 2001), which can oxidize a variety of drugs through many metabolic processes for detoxification(Dresser *et al.* 2000), and it is responsible for about 60% of the metabolism of currently known drugs(Feng *et al.* 2018, Zhou *et al.* 2005). Clinically important CYP3A4 inhibitors include antifungals (e.g. itraconazole and ketoconazole), macrolides (e.g. clarithromycin and erythromycin), antihypertensives (e.g. dihydralazine, verapamil, and diltiazem), anti-HIV drugs (e.g. ritonavir and delavirdine)(Dresser *et al.*2000, Zhou *et al.* 2005). These inhibitors can boost the plasma concentration of itself or other drugs that directly act on hERG and enhance cardiotoxicity(Feng *et al.* 2018, Zhi *et al.* 2015). In the list, ritonavir in lopinavir/ritonavir which has been shown to be effective for the treatment of COVID-19 as a strong CYP3A4 inhibitor can increase the oral bioavailability of certain HIV protease inhibitors, such as lopinavir(Dresser *et al.* 2000).

Potential treatment for COVID-19

According to the guidelines, there are no safe and effective drugs to kill SARS-CoV-2. The validity of the drugs currently in clinical use, including chloroquine, hydroxychloroquine, azithromycin, lopinavir/ritonavir, etc (Table1). are obtained from small clinical trials. Most of these studies have a relatively small sample size, and some of them have the opposite results. Therefore, when prescribing the drugs above, consider not only the results of the current trial but also the patient's condition, as well as possible serious adverse reactions and have careful assessment and choices until a comprehensive and reliable clinical trial is completed.

4.1 Chloroquine and hydroxychloroquine

Chloroquine (CQ) and hydroxychloroquine (HCQ) belong to quinoline antimalarial drugs, among which CQ is the most widely used antimalarial drug in history(Haeusler *et al.* 2018). In the treatment of COVID-19, CQ and HCQ have attracted a great deal of interest. An open-label, non-randomized study involving the application of HCQ (combined with azithromycin in some patients) shows that HCQ treatment is significantly associated with reduced/disappeared viral load in COVID-19 patients(Gautret *et al.* 2020). A small, prospective, observational study also suggests that CQ/CQ ± azithromycin may be efficient on SARS-CoV-2, but the use of these drugs alone or in combination may prolong the QT interval, and therefore increase the incidence of TdP(Saleh *et al.* 2020). In vitro study also found that CQ and HCQ are very effective in controlling SARS-CoV-2 infection(Wang *et al.* 2020), and HCQ is more effective than CQ in inhibiting SARS-CoV-2 in vitro(Yao *et al.* 2020).

There are now more than 100 clinical trials aimed at testing CQ or HCQ against COVID-19 and even CQ and HCQ have been used as standard treatments for COVID-19 patients in hospitals in some countries, such as China(Ledford 2020). Unfortunately, CQ hype is derailing the search for coronavirus treatments, even leading to the treatment available difficultly for patients with autoimmune diseases, which can result in potentially life-threatening manifestations, for instance, lupus nephritis(Jakhar and Kaur 2020, Ledford 2020, Yazdany and Kim 2020). Despite this situation, the data supporting the treatment of COVID-19 with CQ or HCQ is limited(Ledford 2020, Moore 2020, Yazdany and Kim 2020). In the treatment guidelines issued on April 21, the National Institutes of Health noted that there are insufficient data to recommend or oppose the use of CQ and HCQ for COVID-19 populations, and in COVID-19 patients receiving HCQ or azithromycin, 11% -25% of them have excessive QT prolonged to greater than 500 ms(Chorin *et al.* 2020, Panel. 2020). Therefore, better randomized controlled trials of CQ or HCQ are needed to effectively treat COVID-19(Ferner and Aronson 2020).

It is well known that antimalarial drugs have cardiotoxicity(White 2007), and QT prolongation is the most

common adverse reaction(Llanos-Cuentas *et al.* 2014). Although small doses of CQ and HCQ are generally safe, both can block the Kv11.1 potassium channel(Giudicessi *et al.* 2020, Naksuk *et al.* 2020). Long-term use of CQ and HCQ has been reported to induce QT prolongation and malignant arrhythmia(Chen *et al.* 2006, Stas *et al.*2008), chloroquine-induced TdP in a COVID-19 patient also reported(Szekely *et al.* 2020). Antimalarial drug blocking hERG channel has also been found in heterologous expression models and animal models(Sánchez-Chapula *et al.* 2001, Traebert *et al.* 2004). In feline ventricular cardiomyocytes, CQ blocks several inward and outward membrane currents, and the order of potency is inwardly rectifying potassium current (I_{K1})> I_{Kr}> I_{Na}> I_{CaL}(Sánchez-Chapula *et al.* 2001). Besides, CQ also slows the significant rate of hERG deactivation reflecting the dysfunction of drug-bound channels to close.(Sánchez-Chapula *et al.* 2002). On the contrary, as reported by hERG-lite(Wible *et al.* 2005), a novel systematical high-throughput screen for drug-induced hERG risk, CQ increases hERG transport(Borsini *et al.* 2012). The opposite result compared with the reduction of current indicates that there may be a complex mechanism under hERG inhibition induced by CQ. Besides, both CQ and HCQ are metabolized by CYP3A4, the risk of QT prolongation might increase if combined with CYP3A4 inhibitors such as ritonavir/lopinavir or azithromycin(Naksuk *et al.* 2020, Wu*et al.* 2020).

4.2 Azithromycin

Azithromycin, as a macrolide antibiotic, is thought to enhance the therapeutic effect of hydroxychloroquine in COVID-19(Gautret *et al.* 2020). Similarly, a combination of azithromycin and CQ/HCQ was shown to be helpful in the treatment of COVID-19, and there were no reports of death from fatal arrhythmic(Saleh *et al.* 2020). However, considering the cardiotoxicity of antimalarial drugs themselves, whether azithromycin when combined with them increases adverse reactions and whether we should use these drugs alone or in combination to treat COVID-19 is something worth noting. As previously mentioned in a cohort study, the effect of combined azithromycin on prolonging QT interval is more obvious(Mercuro *et al.* 2020), about one-quarter of the QT is prolonged excessively(Chorin *et al.* 2020). Perhaps the azithromycin itself does not usually cause a clinically significant prolongation of QT interval(Thomsen *et al.*2006), but its use in combination with CQ or HCQ may theoretically increase the risk of TdP(Juurlink 2020). These conflicting and poor-quality studies suggest that clinicians should carefully weigh risks and benefits and it may be advisable to avoid these drugs until there is more effective clinical and experimental evidence.

Azithromycin is considered to be the least likely to cause arrhythmia because of the least cardiotoxicity, with an estimated 47 added cardiovascular deaths per one million courses according to the report(Ray *et al.* 2012). Indeed, studies have confirmed that the rank order of arrhythmogenicity is estimated to be erythromycin> clarithromycin> roxithromycin> azithromycin(Milberg *et al.* 2002, Ohtani *et al.* 2000). Specially, azithromycin suppress I_{Kr} current only under the condition of 50 times clinical related concentration (2075 mg/L), and the inhibition rate is about 30%(Zhang *et al.* 2017). And so, not surprisingly, compared with dofetilide proved to be an hERG channel blocker, azithromycin has no same electrophysiological effects and thus azithromycin is said to be safe(Avedissian *et al.* 2019, Thomsen *et al.* 2006). However, based on the evidence of macrolides (such as erythromycin) targeting of hERG channels(Volberg *et al.* 2002), it is necessary to further investigate the interaction of azithromycin with hERG, even though azithromycin mainly increases cardiac Na^+current while only slightly blocks I_{Kr}(Yang *et al.* 2017). Moreover, azithromycin as a weaker CYP3A4 inhibitor than homologous antibiotics may increase the risk of QT prolongation when used in combination with antimalarial drugs in treating COVID-19(Wu*et al.* 2020). This seems to explain that azithromycin in combination with CQ or HCQ has a more significant QT prolongation effect.

4.3 Lopinavir/ritonavir

The HIV protease inhibitor class of antiretroviral drugs has obvious benefits for HIV. As an important CYP3A4 inhibitor, ritonavir can enhance the effect of other protease inhibitors such as lopinavir and atazanavir(Soliman *et al.* 2011, Zhou *et al.* 2005). The combined preparation lopinavir/ritonavir has aroused widespread interest after conducting a clinical trial for COVID-19(Cao *et al.* 2020a). The randomized trial found that for severe COVID-19 patients, lopinavir/ritonavir (400 mg and 100 mg, respectively) treatment does not significantly promote clinical improvement, reduce mortality or reduce the detectability of throat

RNA of SARS-CoV-2, but it is beneficial for some secondary outcomes (e.g. a shorter time to stay in the intensive care unit (ICU)(Cao *et al.* 2020a, Stower 2020). Subsequently, although researchers advocated that lopinavir/ritonavir can be used as an alternative treatment guideline for COVID-19 before the completion of the World Health Organization SOLIDARITY trial(Cao *et al.* 2020b), the side effects including QT duration extension have raised concerns about the higher dose or longer treatment of this program. And based on the available evidence, it is uncertain whether lopinavir/ritonavir and other antiretroviral drugs can ameliorate clinical outcomes or prevent infection in patients with high-risk COVID-19(Ford *et al.* 2020). More recently it has been reported that triple combination of interferon beta-1b, lopinavir/ritonavir, and ribavirin are safer and superior to lopinavir /ritonavir alone in alleviating symptoms in patients with mild to moderate COVID-19(Hung *et al.* 2020). But the same question remains on how to balance the risks and benefits.

The evidence of the HIV protease inhibitor-induced arrhythmia is sufficient(Anson *et al.* 2005, Cao *et al.* 2020a, Gallagher*et al.* 2008, Han *et al.* 2015, Kikuchi *et al.* 2002, Soliman *et al.* 2011, Vicente *et al.* 2019). In vitro, lopinavir, nelfinavir, ritonavir, and saquinavir caused a dose-dependent hERG channel blockade(Anson *et al.* 2005). A randomized clinical trial showed that the treatment with ritonavir-enhanced protease inhibitors and non-enhanced regimens had similar effects on QT duration(Soliman *et al.* 2011), suggesting that combined treatment regimens may be more beneficial with fewer side effects, and for QT prolongation the role of the agent ritonavir as an enhancer might not be so important. Particularly, ritonavir 100 mg does not cause QTc prolongation in healthy subjects(Sarapa *et al.* 2008), so it as a CYP3A4 inhibitor alone may only generate side effects when increasing the blood concentration of related drugs.

Except atazanavir, there is no relevant evidence to support whether other protease inhibitors affect hERG maturation and expression on the plasma membrane(Han *et al.* 2015). It has been confirmed that HIV protease inhibitors induce ERS in intestinal epithelial cells(Wu*et al.* 2010) and ERS can down-regulate cardiac ion channel expression(Liu *et al.* 2018). Referring to the effect of rosuvastatin on hERG(Feng *et al.* 2019), ERS may play an important role in diLQTS. Whether hERG is affected by ERS requires further verification.

Future directions

Various factors can affect the hERG channel to induce QT interval prolongation, and QT prolongation may also be the result of multiple ion channel actions. In the course of COVID-19, in addition to affecting the lungs, causing interstitial pneumonia and severe acute respiratory distress syndrome (ARDS), SARS-CoV-2 also damages multiple organs due to massive inflammatory factors, especially the cardiovascular system, leading to a variety of cardiac problems such as arrhythmia and myocarditis(Guzik *et al.* 2020, Inciardi *et al.* 2020, Zheng*et al.* 2020). In such cases, the cardiotoxicity of relevant therapeutic drugs may be obscured by the disease itself, so it is necessary to conduct comprehensive clinical management to consider whether to use these drugs, and if necessary, monitoring ECG is necessary. Most drug interaction with hERG channel induces a prolonging QT interval that is considered as a major risk factor in pharmaceutical drug development and in addition to hERG channels, drugs acting on many ion channels causing severe arrhythmias have become a limiting factor for their clinical use(Denning *et al.* 2016). Therefore, low cardiotoxicity might be a basic requirement before the drug enters clinical use, which requires a sensitive and effective experimental platform to carry out rigorous in vitro experiments, and it also requires tremendous effort for preclinical management.

In 2013, the U.S. Food and Drug Administration proposed an international initiative termed the Comprehensive *In Vitro* Arrhythmia Assay (CiPA), which recommends that multi-electrode array (MEA) can be used as a measurement tool, combined with human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC -CMs) to conduct a preclinical assessment of the drug to evaluate the risk of TdP. The CiPA initiative requires a comprehensive ion current effect, not just I_{Kr}, to evaluate drug safety, and hiPSC-CMs will write a new chapter of safety assessment guidelines(Denning *et al.* 2016). Compared with heterologous system and animal models in research cLQTS or diLQTS, hiPSC-CMs as an autologous source of the reprogrammed cell shows a huge advantage. Although still immature(Veerman *et al.* 2015), they almost completely reproduce the phenotype of cardiomyocytes in vitro because they contain multiple ion channels that constitute the AP of cardiomyocytes(Ma *et al.* 2011). The results of hiPSC -CMs for proarrhythmia prediction under CiPA

would serve as predictive indicators to the ion channel and in silico modeling prediction of proarrhythmic risk(Blinova *et al.* 2017). More importantly, individual-derived somatic cells with gene mutations can also be reprogrammed to differentiate into disease-specific cardiomyocytes, such as LQTS-hiPSC-CMs(Egashira *et al.* 2012, Itzhaki *et al.* 2011, Portero *et al.* 2017, Sala *et al.* 2019, Wuriyanghai*et al.* 2018) and BrS-hiPSC-CMs(Belbachir *et al.* 2019, Kosmidis *et al.* 2016), from LQTS and Brugada syndrome patients, respectively, which has a considerable significance for studying gene-drug interactions. MEA is a high-throughput screening tool for cellular electrical activity, whose measured field potential duration (FPD) reflects the QT interval and to some extent the activities of various ion channels(Nozaki *et al.* 2014).

Combined with hiPSC-CMs and MEA, many drugs can be screened sensitively and efficiently(Nozaki *et al.* 2014), with great potential and advantages in reducing the cost of drug development compared with the use of immortalized cell lines or animal models. Furthermore, disease-specific hiPSC-CMs can detect effective treatments in vitro that can perform effectively clinical translation(Mehta *et al.* 2018, Schwartz *et al.* 2019). In the future, related work should focus more on systemizing and standardizing hiPSC-CMs/MEA applications for comprehensive preclinical drug safety screening, and generating systematic, large-scale, and available drug safety data to further guide clinical practice. And for those drugs that must be used but have cardiotoxicity including QT prolongation, it is still necessary to further clarify the molecular mechanism behind to try possible rescue strategies. LUF7346 as an hERG channel allosteric modulators was recently shown that it can reverse congenital and drug-induced hERG channel blockade in hiPSC-CMs and heterologous expression models through binding to sites in the channel that are different from traditional drug binding sites to induce a conformational change in the channel(Sala*et al.* 2016). However, an excessively shortened QT interval caused by related rescue strategies is also a problem worthy of attention. The goal of precision medicine may be truly achieved through studies into clinical translation only after a comprehensive assessment of risks and benefits.

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Table 1. Potential therapeutic drugs for COVID-19 and their adverse effect of prolonged QT interval.

Drug name	Effects on COVID-19	Assessments of QT interval	Mechanisms on hERG	References
Chloroquine Hydroxychloroquine	Reduced viral load and inhibition of SARS-CoV-2 in vitro.	QT prolongation resulting in TdP.	Blocking I_{Kr} current. Slowed rate of deactivation and increased transport of hERG by chloroquine.	(Borsini *et al.* 2012, Gautret *et al.* 2020, Saleh *et al.* 2020, Sánchez-Chapula *et al.* 2002, Sánchez-Chapula *et al.* 2001, Yao *et al.* 2020)
Azithromycin	Enhanced Hydroxychloroquine potency of viral elimination.	QT prolongation and increased risk of TdP in combination with Chloroquine or hydroxychloroquine.	Blocking I_{Kr} under high plasma concentration. No evidence of interference with hERG trafficking.	(Chorin *et al.* 2020, Gautret *et al.* 2020, Juurlink 2020, Yang *et al.* 2017, Zhang *et al.* 2017)
Lopinavir/ritonavir	Reduced length of hospital stay for severe patients.	Potential QT prolongation.	hERG channel blockade. No evidence of interference with hERG trafficking.	(Anson *et al.* 2005, Cao *et al.* 2020a)

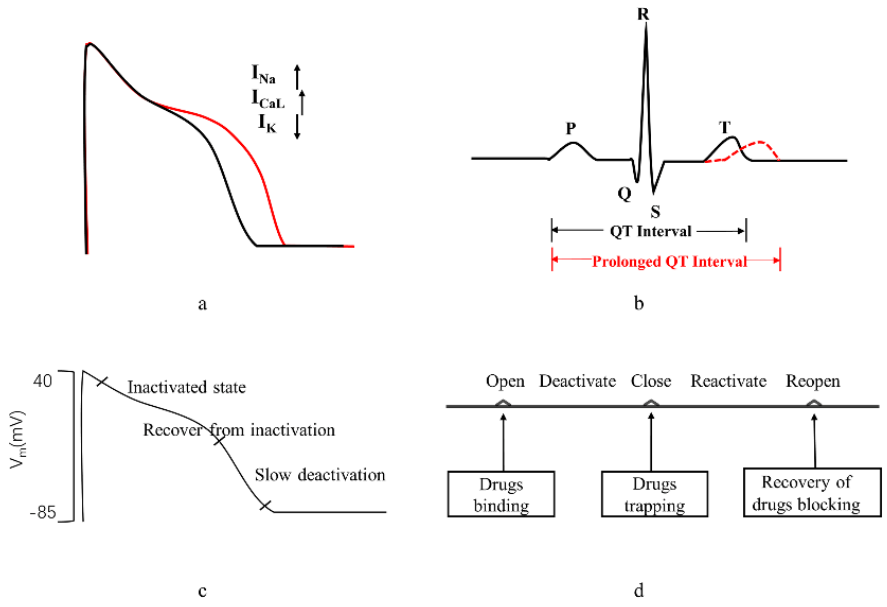

Fig 1. A prolonged action potential duration by increased inward current and weakened outward currents (**a**) and manifested on ECG (**b**). In the early stage of the action potential, the hERG channel experiences a short activation but rapidly inactivates. As depolarization voltage continues to decrease, the hERG reactivates, and then the deactivation rate is much slower to generate the tail currents(**c**). hERG channel opens, with drugs entering the cavity and binding, then the channel closes and the drug is trapped in the cavity. When the channel reopens, the drug blocking effect is released (**d**).

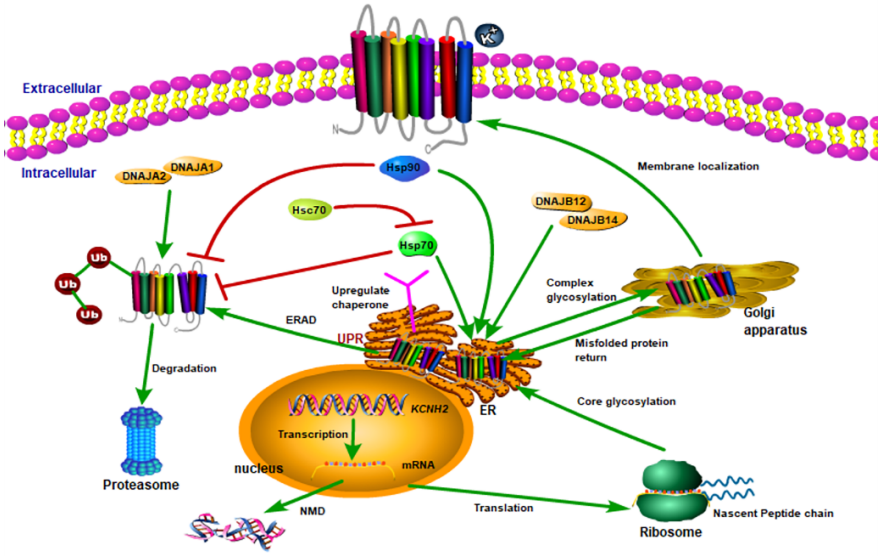

Fig 2. hERG biogenesis and quality control. The *KCNH2*gene is transcribed in the nucleus to generate mRNA. The unqualified mRNA undergoes nonsense-mediated mRNA decay (NMD). The qualified mRNA is translated in the ribosome and is transported to the endoplasmic reticulum (ER) for initial glycosylation. Correctly folded proteins are transported to the plasma membrane to function and misfolded proteins return to the ER to upregulate molecular chaperone levels, including Hsp70, Hsp90, and Hsp40 to regulate protein maturation and degradation (green arrows indicate promotion, the red arrow indicates inhibition). If the misfolded protein persists, it will trigger the unfolded protein response (UPR) making protein enters the ER-associated degradation (ERAD).

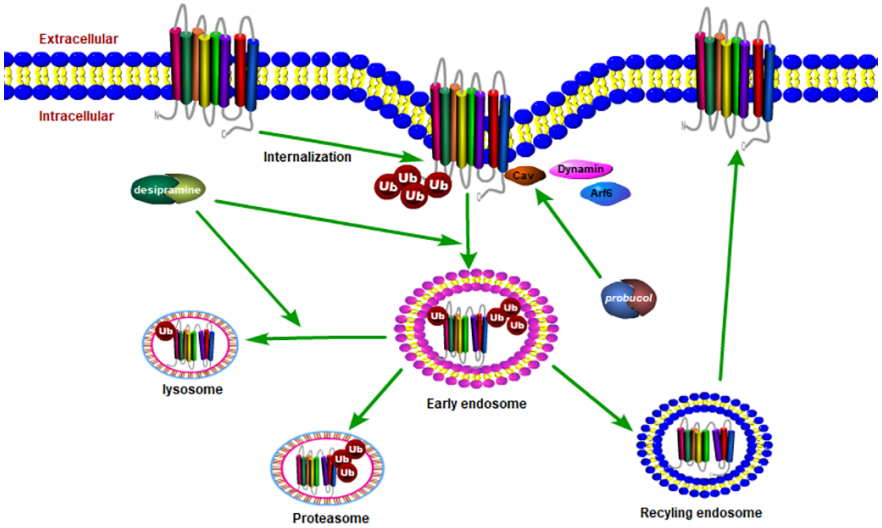

Fig 3. hERG internalization and recycling. hERG channel on the membrane will internalize into clathrin-coated vesicles upon ubiquitination. Caveolin (Cav), dynamin, and Arf6 regulate protein internalization and internalized proteins are degraded by lysosomes or proteasomes, or recycled back to the membrane. Probucol accelerating Cav1 turnover promoting hERG internalization. Desipramine increases channel endocytosis and ubiquitination degradation by the lysosome.

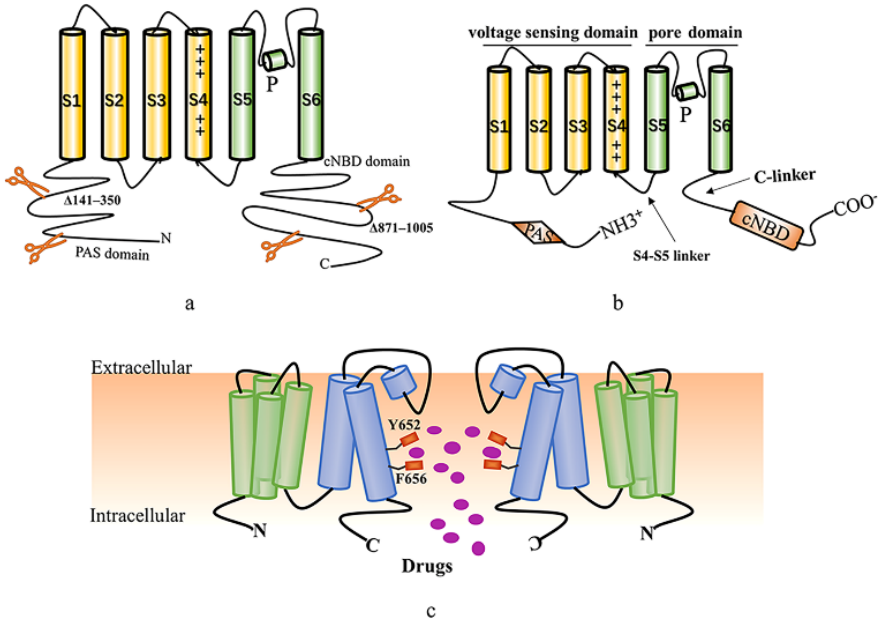

Fig 4. hERG channel structures and drug binding sites. The cryo-EM structure of hERG deleted most of the expected unstructured cytoplasmic regions (Δ141–350 at the N-terminal and Δ871–1005 at the C-terminal) (**a**) and hERG (Kv11.1) channel containing six transmembrane fragments (S1 -S6), the N-terminal Per-Arnt-Sim (PAS) domain, and the C-terminal cyclic nucleotide-binding domain (cNBD). S1-S4 act as transmembrane voltage sensing (VSD) with charged residues and S5-S6 form the pore domain (**b**). hERG channel opens and drugs enter the central cavity. Y652 and F656 on the S6 are two key drug binding sites (**c**).

Competing Interests' Statement: none.