Local Anesthetic Like Inhibition of the Cardiac Na⁺ Channel Nav1.5 by Chloroquine and Hydroxychloroquine

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Introduction: Chloroquine (CQ) and its derivate hydroxychloroquine (HCQ) have been successfully used in the prophylaxis and treatment of malaria for decades.1–3 Besides their antimalarial effect, CQ and HCQ exhibit immunomodulatory and immunosuppressive characteristics and have been established as disease-modifying antirheumatic drugs in the treatment of rheumatic and immunological diseases.4,5 Furthermore, an antiviral effect against several viruses has been demonstrated in vitro and in animal experiments.6–8 Due to the direct antiviral and anti-inflammatory effect, both agents have been proposed for off-label use to treat COVID-19 patients.7,9 Subsequently, after randomized studies were performed without any robust evidence for benefits and uncertainty concerning their safety rose, the FDA retracted an Emergency Use Authorization.10–12 Both agents can cause similar serious side effects ranging from retinopathy, methemoglobinemia or acute kidney injury to life-threatening cardiac arrhythmia.2,11,13,14 QT prolongation, the electrocardiographic
equivalent of delayed ventricular repolarization is the most commonly reported adverse event for CQ and HCQ associated with the treatment of COVID-19 pneumonia.\textsuperscript{15,16} Delayed repolarization facilitates early afterdepolarizations, which can trigger Torsades de Pointes ventricular arrhythmias or even sudden cardiac death. Most of the QT prolongations are induced by drugs and thus classified as acquired long-QT syndrome (LQTS) in contrast to congenital LQTS due to cardiac ion channelopathies. A large proportion of acquired LQTS is triggered by drugs which interact with cardiac hERG K\textsuperscript{+} channels and thereby affect the rapid component of the delayed rectifier potassium current.\textsuperscript{17} Indeed, CQ and HCQ inhibit cardiac hERG K\textsuperscript{+} channels.\textsuperscript{18,19} In addition, both substances have also been demonstrated to inhibit further cardiac ion channels, such as the inwardly rectifying potassium channel, L-type calcium and sodium channels.\textsuperscript{20,21} The opioids methadone or buprenorphine, which can also trigger LQTS, inhibit the cardiac-specific isoform of voltage-gated sodium channels, Nav1.5.\textsuperscript{19,22,23} Vice versa, LAs like bupivacaine, ropivacaine and mepivacaine inhibit hERG K\textsuperscript{+} channels, suggesting that this may contribute to their cardiotoxic potential.\textsuperscript{24} However, inhibition of Nav1.5 is suggested to be the primary mechanism of cardiotoxicity caused by LAs.\textsuperscript{19,25,26} Here we investigated if CQ and HCQ inhibit human Nav1.5 expressed in HEK-293 cells in a manner that may contribute to their cardiotoxicity.

**Materials and Methods**

**Cell Culture and Transfection**

HEK-293 cells were purchased commercially (ECACC 96121229, Sigma-Aldrich, Germany) and cultured at 37 °C and 5% CO\textsubscript{2} in Dulbecco’s Modified Eagle Medium (DMEM), supplemented with 100 U/mL penicillin/streptomycin, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (all GIBCO-Invitrogen, Germany), 3 mM taurine (Sigma-Aldrich, Germany), and 0.4% Zeocin (Invitrogen, Carlsbad, CA). For experiments, either HEK-293 cells stably expressing the human Nav1.5 or HEK-293 cells transfected with Nav1.5-F1760A were used. Mutagenesis of Nav1.5-F1760A was carried out according to the instructions of the manufacturer (QuikChange XL Kit; Qiagen GmbH, Hilden, Germany). Transfection of HEK-293 cells were performed with the Nanofectin transfection kit (PAA Laboratories GmbH, Pasching, Austria) as described previously.\textsuperscript{27}

**Solutions and Chemicals**

The experiments were performed under usage of an external solution that consisted of sterile distilled water and (mmol/l) 70 NaCl, 70 choline chloride, 3 KCl, 1 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 10 HEPES, and 15 glucose. The pH value was adjusted to 7.4 with TMA-OH (tetramethylammonium hydroxide). The pipette solution contained (mmol/l) 140 CsF, 10 NaCl, 1 EGTA, and 10 HEPES. The pH value was adjusted to 7.4 with CsOH. Both solutions were prepared sterile, stored protected from light at 4 °C and used up within 1 month as described before.\textsuperscript{28} Chloroquine was purchased from Hycultec GmbH (Beutelsbach, Germany) and hydroxychloroquine from MedChemExpress (Monmouth Junction, New Jersey, USA). Both agents were prepared as stock solutions to a concentration of 0.1 mol/L according to their data sheets and stored at −80°C. Chloroquine was diluted in dimethyl-sulfoxide (DMSO, Sigma-Aldrich, Munich, Germany) and hydroxychloroquine in distilled water. Directly prior to the experiments, solutions for the patch-clamp recordings were prepared. To achieve the required concentration the appropriate amount of stock solution was titrated with external solution. Solutions were focally applied via a homemade gravity-driven polytetrafluoroethylene-glass application system.

**Whole-Cell Patch-Clamp Recordings and Data Acquisition**

Membrane Na\textsuperscript{+} currents were recorded with the whole-cell configuration of the patch-clamp technique and performed at room temperature as described previously.\textsuperscript{22,28,29} Pipettes for patch-clamp experiments were made of glass capillaries (GB150EFT-10, Science Products, Hofheim, Germany) with a DMZ Universal Puller (Zeitz-Instruments, Germany) and heat polished to achieve a resistance with pipette solution of 2.0 to 2.5 MΩ. Only a single cell was measured per dish and only a single series of measurements was carried out. A patch-clamp amplifier EPC10 amplifier (HEKA Instruments Inc., NY, USA) was operated during all conducted experiments. Its driving program Patchmaster v20 x 60 software (HEKA Instruments Inc., NY, USA) was used for pulse generation but also for data acquisition and to store data obtained from measurement. Currents were filtered at 5 kHz, series-resistance was compensated by at least 60% to minimize voltage
Chloroquine and Hydroxychloroquine Stabilize Fast Inactivation of Nav1.5

Results

Chloroquine and Hydroxychloroquine Inhibit Nav1.5

CQ was shown to block transmembrane Na⁺ currents in primary feline ventricular cardiomyocytes. However, these results were not investigated further regarding the subtype of the voltage-gated Na⁺ channel nor the effect of HCQ on Na⁺ currents. Hence, we examined the capability of CQ and HCQ to inhibit recombinant human Nav1.5 expressed in HEK-293 cells. As demonstrated in Figure 1, both CQ and HCQ induce a concentration-dependent tonic block of resting and inactivated Nav1.5 channels. The behavior of resting channels during exposure to CQ or HCQ was explored in cells held at −120 mV by test pulses to 0 mV applied at 0.1 Hz. The IC₅₀ value for CQ was calculated to 69 ± 12 μM (Hill coefficient 0.96 ± 0.04, n = 9) and for HCQ to 446 ± 50 μM (Hill coefficient 0.97 ± 0.02, n = 9). The difference between CQ and HCQ regarding their blocking potency reaches statistical significance (p < 0.001, unpaired t-test). One of the typical properties of LAs is their high-affinity block of inactivated Na⁺ channels. Induction of inactivation by a 10s long pre-pulse at −70 mV followed by 100-ms-long pulse at −120 mV, allowing recovery from fast inactivation, and finally a test pulse to 0 mV leads to a significant stronger tonic block by HCQ (IC₅₀ 205 ± 20 μM, Hill coefficient 0.86 ± 0.02, n = 8; p < 0.05, unpaired t-test) in respect to the IC₅₀ of resting channels. Tonic block of inactivated channels induced by CQ also results in a lower IC₅₀ value (IC₅₀ 44 ± 9, Hill coefficient 0.97 ± 0.05, n = 12) when compared to resting channels, but this difference did not reach statistical significance (p = 0.62, unpaired t-test).

Chloroquine and Hydroxychloroquine Induce a Use-Dependent Block of Nav1.5

The use-dependent increase in the affinity of LA upon voltage-gated Na⁺ channels, leading to a use-dependent block, explains its clinical importance due to the suppression of hyperexcitability, but could also be relevant with regard to its cardiotoxicity. We questioned if CQ and HCQ induce a use-dependent block of Nav1.5 comparable to LA. Both drugs induce a use-dependent block if Nav1.5 was activated with 60 pulses at 10 Hz in cells held at −120 mV. As it is demonstrated in Figure 2, 10 and 100 μM of CQ induced a current inhibition of 18 ± 0.02% (n = 11) and 33 ± 0.03% (n = 9). HCQ induced a significantly weaker use-dependent block compared to CQ with a reduction of 7 ± 0.01% at 10 μM (n = 10) and 18 ± 0.02% (n = 10) at 100 μM (p < 0.001, unpaired t-test).

Chloroquine and Hydroxychloroquine Stabilize Fast Inactivation of Nav1.5

Various agents with LA-like effects modify the inactivation properties of Nav1.5 channels. Here, were characterized the effects of CQ and HCQ on fast inactivation. Fast inactivation was induced by 50 ms pre-pulses ranging from −120 to 0 mV in steps of 5 mV. The remaining fraction of available channels was examined with a 20 ms long pre-pulse to 0 mV. Figure 3 demonstrates that CQ induced a concentration-dependent shift of the steady-state fast inactivation from Vₐ₀ of −85 ± 0.6 mV (n = 9) in control to Vₐ₀ of −96 ± 1 mV (n = 9) with 10 μM and −102 ± 3 mV (n = 11) with 100 μM. The effects induced by HCQ were similar to CQ (control: −86 ± 0.5 mV, n = 9; 10 μM: −95 ± 0.5 mV, n = 9; 100 μM: −100 ±
1 mV, n = 9). The effect of CQ (100 μM) was not significantly different when compared to HCQ (100 μM) (p = 0.59, unpaired t-test).

Chloroquine and Hydroxychloroquine Do Not Change the Proportion of the Fast and Slow Recovery

LAs are known to exert certain cardiotoxic properties. Previous studies revealed that one of these possible cardiotoxic effects are likely based on a slow dissociation of LAs like bupivacaine and amitriptyline from inactivated channels.\textsuperscript{26,32,33} Accordingly, we investigated if CQ and HCQ share this potential cardiotoxic property with LAs. The time course of recovery from the inactivated state was explored in the presence of 10 and 100 μM CQ or HCQ. Recovery from inactivation was explored via a two-pulse protocol consisting of a 10-s-long inactivating pulse at −70 mV followed by a test pulse to 0 mV whereby the interval between these pulses varied between 0 and 7.3 s.\textsuperscript{27,28} As shown in Figure 4, a biphasic course was obtained that was best fitted with a double exponential fit giving two-time constants (τ1 and τ2). In the presence of a drug, τ1 represents recovery of unblocked channels from a fast inactivation state. τ2 represents recovery of inactivated channels that were blocked during the 10-s pre-pulse, including rebinding and dissociation from resting...
channels and recovery from the slow inactivation state. Application of CQ resulted in a prolongation of both $\tau_1$ (control: 9 ± 0.8 ms, n = 10; 10 µM: 37 ± 2 ms; n = 9; 100 µM: 47 ± 5 ms, n = 7) and $\tau_2$ (control: 379 ± 125 ms, n = 10; 10 µM: 484 ± 67 ms; n = 9; 100 µM: 633 ± 367 ms, n = 7). Administration of HCQ in the same concentrations resulted in a modest prolongation of $\tau_1$ (control: 6 ± 0.4, n = 9 ms; 10 µM: 11 ± 0.8 ms; n = 9; 100 µM: 16 ± 1 ms, n = 8). However, HCQ did not prolong $\tau_2$ (control: 368 ± 108 ms, n = 9; 10 µM: 212 ± 63 ms; n = 9; 100 µM: 229 ± 89 ms, n = 8). In contrast to bupivacaine that unbinds slowly from inactivated channels and therefore increases the fraction of channels recovering with a slow time constant in this protocol, CQ and HCQ did not markedly alter the fraction of channels recovering with $\tau_1$ (~80–85%) and $\tau_2$ (15–20%).

Inhibition of Nav1.5 by Chloroquine and Hydroxychloroquine May Involve the Proposed Binding Sites for Local Anesthetics

Drugs modulate the activity of Nav1.5 by multiple mechanisms. As CQ and HCQ produce a state-dependent inhibition of Nav1.5 which is very similar to the effects of classical LAs, we investigated if inhibition induced by CQ and HCQ involves the established LA binding site of Nav1.5. While multiple residues seem to dictate inhibition by LAs, replacement of F1760 is commonly performed in order to investigate the role of the “LA-binding site”. 34–36
Therefore, we investigated the LA-insensitive mutant channel Nav1.5-F1760A as described by Nau et al.\textsuperscript{33} As displayed in Figure 5, in the presence of CQ Na\textsuperscript{+} currents generated by Nav1.5-F1760A channels exhibited a significantly weaker concentration-dependent tonic block as compared to wild-type Nav1.5 represented by an approximately fivefold higher IC\textsubscript{50} value (IC\textsubscript{50} 330 ± 14 μM; Hill coefficient 0.96 ± 0.01, n = 9; p < 0.01, unpaired t-test). In contrast to CQ, raising concentrations of HCQ exhibited only a slightly attenuated concentration-dependent tonic block and the corresponding IC\textsubscript{50} value was calculated to 666 ± 72 μM (Hill coefficient 0.99 ± 0.02, n = 8; p = 0.17, unpaired t-test). For comparison to the wild-type Nav1.5 channel, the IC\textsubscript{50} value for HCQ was calculated to 446 ± 50 μM. Moreover, in the presence of 10 μM and 100 μM CQ the use-dependent block protocol performed with 10 Hz resulted in less inhibition of Nav1.5-F1760A Na\textsuperscript{+} currents (10 μM: 2.07 ± 0.01%, n=6; 100 μM: 10 ± 0.03%; n = 9; p < 0.01, unpaired t-test) while application of HCQ led to an almost abrogated use-dependent block (100 μM: 0.4 ± 0.02%; n = 8; p < 0.01, unpaired t-test) as compared to wild-type Nav1.5. This experiment indicates that both drugs employ the established LA binding site in order to inhibit Nav1.5.

Figure 3 Chloroquine and hydroxychloroquine stabilize the fast inactivation state of Nav1.5. To induce the fast inactivation state, cells were exposed to 50-ms-long pre-pulses ranging from −120 to 0 mV in steps of 5 mV and the respective fraction of non-inactivated available channels was tested with a 20-ms-long pre-pulse to 0 mV (A and B) Voltage-dependency of fast inactivation of Nav1.5 in the presence of control solution and 10 and 100 μM CQ (A) and HCQ (B). Solid lines represent fits obtained with the Boltzmann equation. (C and D) Representative current traces of Nav1.5 each in the presence of 100 μM CQ (C) and HCQ (D) with their corresponding controls.
Extracellular Alkalinisation Potentiates Inhibition of Nav1.5 by Chloroquine and Hydroxychloroquine

LAs interact with intracellular residues of Na⁺ channels. As LAs are weak bases, alkalinization of the LA-solution increases inhibition of sodium channels due to an increased membrane-permeability. To test if this mode of accessibility also applies for the weak bases CQ and HCQ, the pH-value of the extracellular medium was set to 9.0. Indeed, tonic block of resting Nav1.5 channels was increased by alkalization. The IC₅₀ value for CQ was calculated to 6.8 ± 0.9 μM (Hill coefficient 0.9 ± 0.04, n = 9) and for HCQ to 8.3 ± 0.4 μM (Hill coefficient 1.1 ± 0.04, n = 9).

Discussion

CQ and HCQ have been used for the prophylaxis and treatment of malaria for decades. Furthermore, both substances have been established as disease-modifying antirheumatic drugs in the treatment of different rheumatic and immunological diseases due to their immunomodulatory and immunosuppressive characteristics. However, serious side effects accompany usage of CQ and HCQ, ranging from retinopathy, methemoglobinemia or acute kidney injury to life-threatening cardiac arrhythmia. Especially cardiovascular side effects linked to their administration are reported and frequently the primary cause of mortality.
Figure 5 Chloroquine and hydroxychloroquine may interact with the proposed LA-binding site of Nav1.5. (A and B) Currents of resting Nav1.5 wild-type and Nav1.5-F1760A channels were triggered by 20 ms test pulses from −120 to 0 mV in intervals of 10 s to receive dose–response curves for tonic block by CQ (A) and HCQ (B). Peak amplitudes of Na⁺ currents at different drug concentrations were normalized with respect to the peak amplitude in control solution and plotted against the concentration of CQ (A) and HCQ (B). Data were fitted with the Hill equation as indicated by the solid line. (C and D) Development of use-dependent block of Nav1.5 wild-type and Nav1.5-F1760A channels by 100 µM CQ (C) and 100 µM HCQ (D). Peak currents were normalized to the amplitude of the first pulse and plotted against the pulse number. (E and F) Representative traces of currents generated by Nav1.5-F1760A for the dose–response curves of CQ (E) and HCQ (F). (G and H) Representative current traces of Nav1.5-F1760A activated at 10 Hz in the presence of 100 µM CQ (G) and HCQ (H).
CQ and HCQ are considered as Vaughan Williams Class Ia antiarrhythmics because of their structural similarity and comparable antiarrhythmic properties to quinidine.\textsuperscript{41,42} Class I agents inhibit both peak and late sodium currents by blocking Nav1.5, a feature previously described for CQ and HCQ.\textsuperscript{21,43,44} Blockade of Nav1.5 is associated with an intracardiac conduction delay and thus, depending on the extent of the propagation delay, with an elongation of the QRS complex in the electrocardiogram.\textsuperscript{45,46} Drug-induced QRS prolongation due to Nav1.5 inhibition which slows the rate of cardiac depolarization enables life-threatening ventricular arrhythmias and even sudden cardiac death, thus contributing to morbidity and mortality associated with Nav1.5 blockers.\textsuperscript{46,47} In terms of cardiac toxicity, Nav1.5 is a critical target for LAs.\textsuperscript{25,28} Previously, for other cardiotoxic drugs with arrhythmogenic properties including buprenorphine, methadone and amitriptyline, LA-like effects on Nav1.5 were described.\textsuperscript{19,22,29,32,48} In fact, CQ exerts an LA-like pain suppressing effect when injected intradermally or subcutaneously, thus providing immediate analgesia like classical LAs, indicating possible LA-like effects also on Nav1.5.\textsuperscript{49} This in vitro study was designed to explore whether CQ and HCQ exert LA-like effects on the cardiac voltage-gated Na\textsuperscript{+} channel Nav1.5 as a feasible target for their proarrhythmic properties. We were able to demonstrate that both drugs are concentration- and state-dependent inhibitors of Nav1.5. Furthermore, the LA-insensitive mutant Nav1.5-F1760A displayed a reduced sensitivity to both substances. Thus, our data suggest that CQ and HCQ indeed have LA-like properties and even employ the proposed intracellular LA-binding site. However, prolongation of the QT interval leading to acquired LQTS has been primarily explained by the interaction between CQ and HCQ with cardiac hERG K\textsuperscript{+} channels and not linked to Nav1.5.\textsuperscript{51,42,46} Furthermore, various sodium channel blockers like the LA lidocaine, the antiarrhythmics mexiletine and flecainide, but also CQ, have been shown to suppress persistent sodium currents which are associated with congenital LQTS-type-3 and thereby may partially offset the QT prolongation that is induced through blockade of hERG K\textsuperscript{+} channels.\textsuperscript{42,50} In addition, in an analysis of pooled congenital LQTS patients by Yang et al mexiletine and flecainide as representatives of class Ib and Ic antiarrhythmics, significantly shortened the corrected QT time.\textsuperscript{51} Although this property of various sodium channel blockers mediate an antiarrhythmic effect, the use of CQ and HCQ is accompanied by a widened QRS complex and a prolongation of the QT interval as a commonly reported side effect, as recently reported in patients with COVID-19 and concomitant use of CQ or HCQ.\textsuperscript{52,53} Accordingly, in contrast to class Ib and Ic antiarrhythmics, the clinical significance of blocking late sodium currents in terms of QT interval shortening by both agents seems to be negligible. Moreover, it should be considered that Nav1.5 blockade by CQ and HCQ could secondarily affect repolarization via a delay in the course of depolarization, thus rather contributing to a prolonged QT interval.\textsuperscript{45} Hence, our results provide a more detailed understanding of how modulation of Nav1.5 channels by CQ and HCQ might contribute to their arrhythmogenic effects.

LAs inhibit Na\textsuperscript{+} channels by locking the pore from the intracellular side during the open state and are therefore described as use-dependent open state block.\textsuperscript{37} At physiological pH-values, most LAs are predominantly positively charged due to protonation and have to be deprotonated into their neutral form before crossing the cell membrane. Alkalization of the extracellular medium changes the ratio between the two states in favor of the deprotonated form leading to an increased membrane permeability and pharmacological effect.\textsuperscript{38,54} The IC\textsubscript{50} values for inhibition of Nav1.5 by CQ and HCQ were reduced as well, supporting our notion that both substances inhibit Nav1.5 by binding to an intracellular site. Along with the findings that CQ and HCQ induce a use-dependent block and may bind to the proposed binding sites for LA, these results suggest that both agents have to be deprotonated to pass the cell membrane before reaching their binding site.

CQ and HCQ have similar pharmacokinetics. After application, sequestration across all tissues is an important attribute, whereby only clinically unimportant differences in tissue distribution of CQ and HCQ were observed.\textsuperscript{55,56} Preclinical experiments revealed that the tissue/plasma CQ-concentration ratio in the heart after an intraperitoneal dose vary between 6.8 after 1 hour and 20 after 4 hours, indicating that CQ-concentrations are higher in heart tissue than in the plasma.\textsuperscript{57} These results were confirmed in different animal and human studies.\textsuperscript{56} Steady-state plasma concentration of CQ during therapy of rheumatoid arthritis generates plasma concentrations of 1–3μmol/L.\textsuperscript{58} In patients with long-term use of CQ, serum concentrations above 2.5μmol/L are frequently associated with typical side effects.\textsuperscript{59} HCQ was reported to be less toxic but safety concerns have also been raised for cardiac toxicity.\textsuperscript{7} However, a meta-analysis of randomized trials found an increased mortality in HCQ-treated groups for therapy of COVID-19.\textsuperscript{60} Due to their
comparable tissue distribution and reported cardiac toxicity in prevention and treatment of COVID-19, clinically relevant levels of CQ and HCQ seems to be close to the concentration range of CQ and HCQ which may cause effects on Nav1.5.

In general, the IC$_{50}$ value is an accepted value to describe the inhibitory potency of a substance. The IC$_{50}$ values for resting Nav1.5 channels yielded from our experiments were about 69 μM for CQ and 446 μM for HCQ. Jordaan et al found somewhat divergent IC$_{50}$ values for CQ (8 μM) and HCQ (>300 μM). In contrast, Thomet et al stated that on ATX-II-induced non-inactivating persistent currents of Nav1.5, inhibition by CQ and HCQ gave IC$_{50}$ values of 159 μM and 96 μM respectively. The IC$_{50}$ values for the rapid component of the delayed rectifier potassium current mediated by hERG K$^+$ channels are considerably lower (2–8 μM for CQ and 3–10 μM for HCQ). However, the sole validity of IC$_{50}$ values for predicting proarrhythmic risks due to inhibition of hERG K$^+$ channels have been challenged, noting that they do not account for dynamic time- and state-dependent drug–channel interactions. Furthermore, the IC$_{50}$ value is not necessarily related to the arrhythmogenic potential. Both CQ and HCQ also inhibit L-type calcium currents with IC$_{50}$ values of 3–30 μM and 8–90 μM, respectively. Kir2.1, generating the rectifying potassium current, is also blocked by both agents with IC$_{50}$ values of 6 μM for CQ and 30 μM for HCQ. Taken together, different studies confirm that both CQ and HCQ inhibit several cardiac ion channels with potential significance for cardiac arrhythmias.

Resting Nav1.5 channels appear to require high concentrations of both substances to get inhibited, thus the relevance for their arrhythmogenic potential remains unclear. Resting potentials in mammalian hearts depend on the type of cells and varies between −50 mV in sinusatrial node cells and −90 mV in ventricular cells. Thus, recording currents of resting channels held at hyperpolarized potentials of −120 mV as applied in this study is artificial and not representative for the membrane properties of cardiomyocytes in vivo. Since the physiological transmembrane resting potential of mammalian hearts lays around −90 mV in ventricular cells and, due to an ongoing cardiac activity, different conformational states of Nav1.5, the clinical relevance of inhibitors should also be measured from recordings on inactivated channels. Indeed, our data show that both CQ and HCQ exert an LA-like high-affinity block of Nav1.5 channels due to an enhanced binding to the inactivated state. Another important property of LA regarding their cardiotoxicity may be use-dependent inhibition. For example, bupivacaine known for its high cardiotoxic potential induces a very strong use-dependent block. Sánchez-Chapula et al demonstrated that CQ blocked several ion currents in primary feline ventricular myocytes, including Na$^+$ currents. However, 10 μM CQ did not induce a use-dependent block. In contrast, our experiments suggest that CQ and HCQ induce a modest use-dependent block of Nav1.5 using a frequency concordant to previous studies. Furthermore, CQ induced a stronger use-dependent block as compared to HCQ. This difference between CQ and HCQ correlates with their different cardiotoxicities. Nevertheless, both agents induce less use-dependent block compared to bupivacaine and other LAs but showing similar effects to methadone which was reported to have relevant LA-like properties.

In addition to the use-dependent block as a surrogate for an increasing affinity of Nav1.5 channels towards an agent as a response to repetitive depolarizations, substance-specific kinetics are responsible for the duration of their blocking capabilities. Fast onset of inhibition and slow dissociation from inactivated cardiac Na$^+$ channels of bupivacaine and amitriptyline led to the generally used classification of “fast in” and “slow out” blockers. Due to slow dissociation of agents with inhibiting properties from inactivated Na$^+$ channels, the vast majority of channels may remain in an inactive state between two action potentials, resulting in an accumulation of agent-bound inactivated channels and finally to cardiac arrhythmias. However, neither CQ nor HCQ exhibit effects similar to those of bupivacaine or amitriptyline. CQ and HCQ only exhibit a modest change of the proportion of the fast and slow recovery components. This indicates that both CQ and HCQ appear to dissociate quite fast from inactivated channels, a property they share for example with lidocaine and therefore do not readily explain their cardiotoxicity.

**Conclusion**

Both CQ and HCQ can cause ventricular arrhythmias and sudden cardiac death by affecting ventricular depolarization and repolarization, which manifest as prolongation of the QRS complex and the QT interval, latter classified as acquired LQTS. Furthermore, several studies suggest that these mechanisms are caused by a multiple ion channel block in cardiac cells. In this study, it was demonstrated that both agents modify Nav1.5 and exhibit LA-like properties. Our experiments on LA-insensitive Nav1.5-F1760A channels suggest that CQ and HCQ bind to the proposed LA-binding site to inhibit...
Nav1.5. We conclude that this study identified an additional molecular mode of action for the proarrhythmic properties of CQ and HCQ.

**Abbreviations**

LA, local anesthetic; hERG, human ether-à-go-go-related gene; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; FDA, US Food and Drug Administration; LQTS, long-QT syndrome; HEK-293 cells, human embryonic kidney-293 cells.

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**Disclosure**

The authors report no conflicts of interest in this work.

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