Hydroxychloroquine's Influence on Hypoxic and Hypokalemic ventricle: An Insilico Perspective

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Abstract

Hydroxychloroquine (HCQ) has been widely used, irrespective of pre reported cardiotoxicity. This demands further investigation on the mechanisms of HCQ interaction under hypoxia without and with a pro-arrhythmic comorbidity like hypokalemia in the ventricular tissue as well as its effects when excited with premature beats (PBs) to understand the possibility of arrhythmic occurrence. This is made possible by configuring a 2D transmural anisotropic ventricular tissue model consisting of endocardial, midmyocardial and epicardial myocytes for mild and severe hypoxia, hypokalemia and HCQ conditions. Results show that along with a QT interval reduction, low amplitude or T-wave inversion is observed in mild and severe hypoxia conditions respectively. No significant adverse effect of HCQ is observed in both cases. Under hypokalemia, mild hypoxia creates notched T-waves. Including HCQ has the effect of increasing the QT interval and T-peak. In presence of PBs, arrhythmia is generated only in presence of hypokalemia. Further, severe hypoxia causes inverted T-waves and a shortened QT-interval in hypokalemic comorbid configuration. In presence of PBs, reentry is created only on addition of hypokalemia. When treated with HCQ, no notable changes occurred. This in-silico ventricular model indicates that HCQ treatment has no significant adverse effect in presence of hypokalemia and hypoxia, except in the combination of mild hypoxia with hypokalemia condition where it initiated a re-entrant arrhythmia pattern. These results could help guide treatment with HCQ.

1. Introduction

Although Hydroxychloroquine (HCQ) is primarily labelled as an antimalarial drugs, it has widespread benefits in treating other autoimmune diseases [1]. Recently, HCQ gained popularity as a choice of treatment for the novel severe Acute Respiratory Syndrome Coronavirus (COVID-19)[2], but was later discontinued.Findings from previous studies suggested that, long-term intake of HCQ is linked to development of QRS widening, QT interval prolongation, ventricular arrhythmia like Torsades de pointes (TdP), hypokalemia and hypotension [3]. In contrary, experiments performed on mouse atria by Capel et al. [4] reported that HCQ acted as a bradycardiac agent in sinoatrial cells via a dose-dependent reduction of multiple ionic currents. Chan et al. reported that, severity of malarial infection influences the shortening of QT interval and sensitivity to changes in heart rate [5]. Furthermore, clinical observations reported by Mercuro et al. [6] shows baseline QT_c was 455 ms in 90 COVID-19 patients and it increased to 473 ms in presence of HCQ. Among those who received HCQ, 19% had QT_c prolongation of 500 ms or more, 13% had a change in QT_c of 60 ms or more and 1 case of Torsade de Pointes (TdP) was reported. Thus its very evident that investigating and understanding the cardiac manifestation mechanism due to medication like HCQ is critical. Hypokalemia is recognised as a common complication of severe malaria. Also, Li X et al.'s study on 175 patients with COVID-19 reported varying intensities of hypokalemia [7]. Hypoxaemia or oxygen saturation has been used as a predictor for death in malaria infected children [8]. He et. al [9] proposed that ACE-2 signalling pathways may play a role in cardiac injury while hypoxemia caused by COVID-19 may cause damage to myocardial cells.

Although researchers have attempted to study the pharmacokinetics of HCQ: it is difficult to narrow down the inhibitory mechanism of a drug on human cells under normal conditions and specific abnormal pathologies. Computational models help bridge this gap, by understanding the effect of HCQ on a hypoxic ventricle and in presence of a comorbidity like hypokalemia. This is done by using a 2D ventricular tissue model, to understand the cardiac mechanism, including the response to pharmacological agents like HCQ. Two variations of hypoxia; mild and severe are explored. Hypokalemia is then introduced to understand its effect on ventricular tissue. In each case, the variations in QT interval and T-peak are recorded. Finally, the tissue is excited with premature stimuli to analyse under which of the above conditions the tissue becomes pro- arrhythmic.

2. Methods

In this study, we build on Priya et al.'s 2D anisotropic transmural ventricular model made up of endocardial (endo), midmyocardial (mid) and epicardial (epi) layers [10]. The change in cardiomyocyte's ionic current parameters in the various configurations: hypokalemia and hypoxia are summarised in Table 1.

Table 1: Change in parameters for different conditions.

Condition	Ionic Current	Change
HCQ	I_{Kr}	35% reduction
	I_{CaL}	12% reduction
Hypokalemia	K_o^+	55% reduction
Mild Hypoxia	$[ATP]_i$	5.5 mM
	$k_{0.5}$	0.125
Severe Hypoxia	$[ATP]_i$	5 mM
	$k_{0.5}$	0.250

As COVID-19 or Malaria have been linked to causing hypoxemia, which in turn leads to hypoxia, this condition was included in the cardiac myocytes by increasing intracellular ATP concentration which would in turn lead to activation of an ATP sensitive potassium current. The formulation of Shaw and Rudy [11] is used to describe ATP activated K^+ current where $G_{k,ATP}$, the maximum conductance of I_{ATP} current is set to a value of 3.9 nS/cm^2, H and n are set to 2 and 0.24 respectively. The intracellular ATP concentration ($[ATP]_i$) under normal condition is 6.8 mM, but it decreases to 5.5 mM in mild hypoxia and 5 mM in severe hypoxia respectively. Similarly, $k_{0.5}$ is 0.042 for normal condition, 0.125 and 0.25 for mild and severe hypoxia respectively [12].

To investigate the benefits and adverse effects of HCQ under control, hypoxia and hypokalaemia pathologies, the ion channel variations corresponding to these conditions were included in the cells of the tissue one at a time. HCQ drug has been reported to reduce the rapid delayed rectifying potassium current (I_{Kr}) and I_{CaL} by 35% and 12% respectively in atrial cells [4]. The same modifications have been considered for our model. In addendum, hypokalaemia is introduced in the cells by reducing the extracellular potassium concentration (K_o^+) to 55%. A regular pacing pulse of 800 ms (corresponding to 75 beats per minute) is applied in the tissue. In addition, premature stimuli is introduced in between the normal beats to study which conditions can initiate or sustain an arrhythmia. The tissue depolarisation and repolarisation patterns generated under various conditions are validated by simulating pseudo ECGs.

3. Numerical Results and Discussion

The lower leftmost corner (cells 1:10,1:2) of the developed transmural tissue framework of cardiomyocytes is stimulated by a current of 52 μA for 1 ms. As a result, a convex wavefront propagates from the endo to mid and epi layer from the bottom to the top of the tissue. The repolarisation occurs first in the epi and endo layers, and M-cells in the mid layer are the last to repolarise. Normalised pseudo ECGs are synthesized from this tissue. *Mild* and *severe* hypoxia conditions are introduced in the tissue to study its effect without and with HCQ. Furthermore, hypokalemia is included to understand the its influence. In each of these conditions, the QT interval and T-peak amplitudes are recorded in Table 2.

Table 2: QT interval and T-peak for different conditions.

Condition	QT interval (sec)	T-peak (mV)
Control	0.345	0.2265
Control with HCQ	0.365	0.262
Mild hypoxia	0.325	0.152
Severe hypoxia	0.275	-0.170
Mild hypoxia with HCQ	0.340	0.181
Severe hypoxia with HCQ	0.275	-0.153
Hypokalemia & Mild hypoxia	0.355	0.141
Hypokalemia & mild hypoxia with HCQ	0.38	0.174
Hypokalemia & Severe hypoxia	0.300	-0.156
Hypokalemia with severe hypoxia with HCQ	0.305	-0.126

Addition of HCQ in control conditions increases the QT interval and the T-peak by 5.85% and 15.67% respectively. In contrast, the QT interval shortens by 5.79% and T-peak decreases by 33.33% under mild hypoxia. On including HCQ drug, QT interval slightly increases by 1.45% and T-peak rises by 20.08% in comparison with no HCQ. However, it doesn't reach the control values. Under severe hypoxia conditions, the QT interval is further reduced by 20.29% and a negative T-wave peak of -0.17 mV is observed along with a QT depression. This negative T-peak might be representative of ischemia in clinical ECG recordings. In contrast, the effect of HCQ in *severe* hypoxia is negligible. Here, a pacing interval of 800 msec (i.e HR is 75 beats/min) is considered, so the Bazett QT_c interval is 0.363 sec and 0.307 sec in *mild* and *severe* hypoxia conditions. On adding HCQ, the QT_c interval is in increased to 0.380 sec in *mild*, while it remains the same in *severe* hypoxia conditions. The QT_c values reported in our study are lower than those observed clinically by Mercuro et al. [6] due to the limitation of considering only a segment of the ventricle. However, the percentage increase in APD between control and HCQ in their study is

3.95% which matches closely to the percentage increase of 4.68% in QT_c observed here in *mild* hypoxia with HCQ condition.

Under hypokalemia and *mild* hypoxia, the QT interval increases by 2.89% while T-peak reduces by 37.74% in comparison to control. On including HCQ, the QT interval is prolonged by 10.14% and the T-peak is reduced by 23.17%. In the combination of hypokalemia and *severe* hypoxia, a negative T-peak of 0.156 mV is observed with reduced QT interval of 13.04%. Here too, HCQ has no significant effect, other than a slight increase in the QT interval and T-peak to 0.305 s and 0.126 mV respectively. Thus, it can be summarised that severe hypoxia can proliferate risk due to the presence of inverted T-wave, that implies occurrence of ischemia.

3.1. Cardiac tissue response in presence of hypoxia, and when treated with HCQ

To analyse arrhythmia occurrence, the cardiac tissue is paced with premature beats (PBs) in presence of normal pacing beats of 800 ms. Three consecutive PBs are applied after the first normal pacing pulse, to enable creation of an arrhythmic pattern (as single or two PBs were not found to be effective in creating an arrhythmia). In each case, the duration of PBs is determined by the time the endo cells located at the bottom of the tissue have come out of their refractory state and are re-excitable again. Initially, PB pacing is tested for *mild* and *severe* hypoxia configurations and then HCQ is included in the cells of the tissue, to understand if it can give rise to an arrhythmia. Finally, hypokalemic condition is added to study its influence.

Fig.1 shows the pseudo ECG on including *mild* and *severe* hypoxia conditions in the tissue and on HCQ exposure. Under *mild* hypoxia, three PBs each of 295 ms duration are applied after the first beat. The mid cells in the tissue are in a repolarising state when the first PB is applied. This causes the depolarisation from the first PB to travel upward along the endo layer and later depolarise the mid and epi layer. Repolarisation occurs from the endo, mid and then epi layer which appears as a negative T-wave in the ECG. The depolarisation wavefront from the second PB is not able to excite the cells in the epi layer as they are in a refractory state and this appears as a ST segment elevation. Further, when the third PB occurs, an inverted T-wave is created as the mid and epi cells repolarise simultaneously. Later, normal pacing pulses are resumed at 1.6 s in both the cases. Presence of HCQ shows a similar trend. Our study is in line with those reported by Wang et al.[13], where a dosage of 10 mM HCQ prolonged the APD of cells but didn't induce an arrhythmia in tissue.

In *severe* hypoxia, application of three PBs, every 250 ms, leads to an ECG pattern with increased negative amplitude T-peak due to changes in depolarisation and repolarisation pattern, same as that of the first PB in mild hypoxia case. The negative T-peak amplitude of first and third PBs is more than that of second PB. Normal ECGs are resumed at 1.6 sec in both scenarios. Thus, it can be inferred that an inverted T-wave morphology (respresentative of ischemia) can be used as a bio-marker for *severe* hypoxia conditions. Further, HCQ drug causes negligible modifications in the voltage propagation patterns and no effect has been observed in the ECG compared with control.

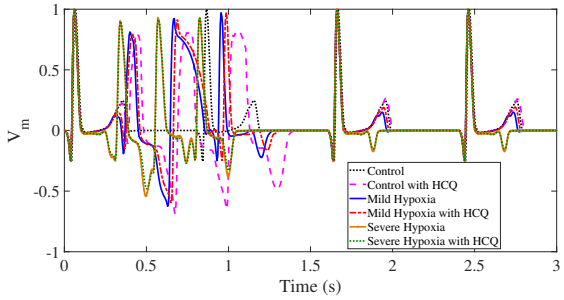

Figure 1: Pseudo ECG for Control, Mild and Severe hypoxia and when treated with HCQ

3.2. Cardiac tissue response in presence of Hypokalemia and Hypoxia, and when treated with HCQ

The influence of hypokalemia is analysed by including it in the tissue and recording pseudo ECGs by pacing the tissue with PBs in the presence of varying severity of hypoxia and adding HCQ.

Hypokalemia and *mild* hypoxia conditions are included in the tissue and paced with PBs as shown in Fig.2. The tissue is regularly paced every 800 msec. Reentrant activity is observed in the pseudo ECG after pacing with three PBs each of 300 msec duration from 0.355 sec to 0.935 sec as seen from voltage maps in Fig. 2. On adding HCQ and pacing the tissue with 3 PBs, each of 330 msec duration, in between the regular pacing interval of 800 msec, although arrhythmic-like activity is observed from 0.37 sec to 1.295 sec in the pseudo ECG, this is due to the depolarisation and repolarisation sequence of the cells in the tissue and not because of reentry. Normal beats resume from 1.6 sec.

In case of *severe* hypoxia conditions, three PBs are applied after every 250 msec. The excitation of the first and third PB appears as an ST-elevation. On introducing HCQ, a similar ECG waveform is observed and no reentry of wavefront is observed.

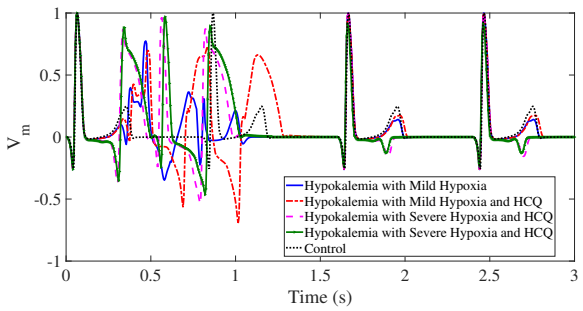

Figure 2: Pseudo ECG for hypokalemia with *mild* and *severe* hypoxia, and when treated with HCQ

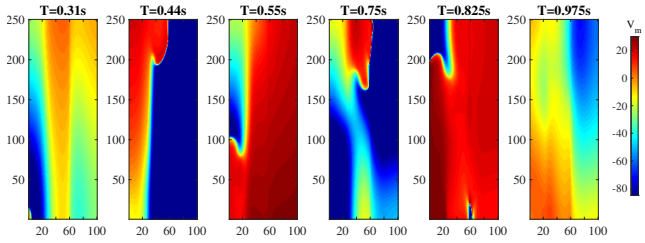

Figure 3: Voltage maps showing reentry in hypokalemia with *mild* hypoxia condition.

4. Conclusion

This study presents the complete electro physiological mechanism of a hypoxic ventricle with a comorbidity, hypokalemia at the tissue level and its responses to HCQ treatment. This model strategically allows more direct studies of ion channel perturbation from clinical observation of infected victims. The main conclusion of this study is, a hypoxic cardiac tissue engenders shorter QT interval, low amplitude or inverted T-waves and ST depression; which could be used as biomarkers. When treated with HCQ, in case of severe hypoxia, there is no significant adverse effect, but in mild hypoxia, QT interval prolongs and T-peak increases in ECG. In particular, the hypokalemic ventricle is prone to arrhythmia, in presence of hypoxia. However, in all hypokalemic conditions, HCQ drug has no significant effects on cardiac ventricle. Thus, these finding could be considered for HCQ management in patients with pre-existing pathologies.

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