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BASIC SCIENCE

Modelling sudden cardiac death risks factors in patients with coronavirus disease of 2019: the hydroxychloroquine and azithromycin case

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Aims Coronavirus disease of 2019 (COVID-19) has rapidly become a worldwide pandemic. Many clinical trials have been initiated to fight the disease. Among those, hydroxychloroquine and azithromycin had initially been suggested to improve clinical outcomes. Despite any demonstrated beneficial effects, they are still in use in some countries but have been reported to prolong the QT interval and induce life-threatening arrhythmia. Since a significant proportion of the world population may be treated with such COVID-19 therapies, evaluation of the arrhythmogenic risk of any candidate drug is needed.

Methods and results Using the O'Hara-Rudy computer model of human ventricular wedge, we evaluate the arrhythmogenic potential of clinical factors that can further alter repolarization in COVID-19 patients in addition to hydroxychloroquine (HCQ) and azithromycin (AZM) such as tachycardia, hypokalaemia, and subclinical to mild long QT syndrome. Hydroxychloroquine and AZM drugs have little impact on QT duration and do not induce any substrate prone to arrhythmia in COVID-19 patients with normal cardiac repolarization reserve. Nevertheless, in every tested condition in which this reserve is reduced, the model predicts larger electrocardiogram impairments, as with dofetilide. In subclinical conditions, the model suggests that mexiletine limits the deleterious effects of AZM and HCQ.

Conclusion By studying the HCQ and AZM co-administration case, we show that the easy-to-use O'Hara-Rudy model can be applied to assess the QT-prolongation potential of off-label drugs, beyond HCQ and AZM, in different conditions representative of COVID-19 patients and to evaluate the potential impact of additional drug used to limit the arrhythmogenic risk.

Keywords COVID-19 • QT duration • Arrhythmia • Predictive model • Asymptomatic

Introduction

The coronavirus disease of 2019 (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and first identified in Wuhan, China, in December 2019, has rapidly become a global pandemic, with >112 million confirmed cases and over 2 490 000 deaths on 25 February 2020 (WHO COVID-19 Dashboard). The high transmission rate of the virus and the lack of collective immunization and therapy have made it a threat to public health, despite its low morbidity in a large part of the population.[1]

Pre-existing cardiovascular disease, including cardiac arrhythmias, is associated with a prognosis worsening.[2–5] Arrhythmias were reported in 17% of patients affected by COVID-19 and this percentage reaches 44% for patients in intensive care unit (ICU).[2–4] In the absence of approved drugs to prevent or treat COVID-19, many clinical trials have been initiated to test the efficiency of drugs already approved for other diseases on this new pathology. Among those, >260 focused on hydroxychloroquine (HCQ, clinicaltrials.gov), a chloroquine (CQ) derivative historically used to treat malaria and autoimmune diseases.[6] Hydroxychloroquine has shown potent *in vitro*

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What's new?

- O'Hara-Rudy (ORd) computer model can be used to assess, at the electrocardiogram level, coronavirus disease of 2019 (COVID-19) off-label drug pro-arrhythmic potential in conditions when the repolarization is impaired.
- Patients with impaired repolarization reserve are at high risk of arrhythmias with such treatments.
- ORd model may help select anti-arrhythmic therapy in addition to COVID-19 treatments.

activity against both SARS-CoV-1 and SARS-CoV-2.[7–9] Two small, non-randomized, open-label clinical trials in France, suggested that the combination of HCQ and azithromycin (AZM) drugs may reduce the viral load of infected patients and improve clinical outcomes.[10,11] Despite accumulation of studies questioning the clinical efficacy of HCQ, the topic was highly debated.[12–18]

Hydroxychloroquine has been occasionally reported to prolong the QT interval on surface electrocardiogram (ECG) and provoke *torsades de pointes* (TdP), a life-threatening arrhythmia.[19–23] AZM has been developed for the treatment of respiratory tract infections[24–26] because the related macrolide, erythromycin, induced prolonged QT intervals, and TdP. Nevertheless, AZM has been occasionally reported as a triggering factor of QT prolongation,[27,28] arrhythmias[26,29,30], and increased risk for sudden death.[26,31,32] Both HCQ and AZM are categorized as being at '*torsades de pointes*' risk (crediblemeds.org) and their administration is not recommended to patients presenting with congenital long QT syndrome (LQTS).[33] On the other hand, large population studies indicate that AZM use was not associated with an increased risk of death from cardiovascular causes in a general population of young and middle-aged adults,[34] and 85 outpatients treated with HCQ for connective tissue diseases for a minimum of 1 year did not show QTc interval and heart rate different from those in a population of healthy young adults.[35] Last, in two recent studies investigating HCQ and AZM treatment of COVID-19 patients, subsets of 9.2% (11/119 patients) and 16% (40/251) of the treated patients presented severely prolonged QTc to values >500 ms, a known marker of high risk of malignant arrhythmia and sudden cardiac death.[36,37] A more recent meta-analysis reported major QTc prolongation >60 ms in ∼13% of the COVID-19 patients treated with both drugs, with an overall considerable heterogeneity, though.[38]

In face of this variability, we exploited a computer model of human ventricular wedge to test the arrhythmogenic potential of a combination of several factors: (i) HCQ and/or AZM treatments, (ii) events occurring in COVID-19 patients that can contribute to alter repolarization: hypokalaemia, tachycardia, and (iii) subclinical LQTS phenotypes. We chose the O'Hara and Rudy pseudo-ECG computer model, based on non-diseased human ventricular data.[39] This model has been previously used and thoroughly validated by many laboratories, including ours, to study cardiac pathophysiological mechanisms in multiple diseases such as inherited and acquired long QT, short QT, and Brugada syndrome.[40–47] The model was adapted to incorporate off-target effects of HCQ and AZM on cardiac ion currents.[28,48]

Methods

Transmural wedge simulations

We computed the pseudo-ECG using a one-dimensional model of a transmural wedge consisting in 165 human ventricular myocytes [O'Hara-Rudy (ORd) model].[39] Cells 1–60 were sub-endocardium type, 61–105 were mid-myocardium type, and 106–165 were sub-epicardium type (Supplementary material online, *Figure S1*). The spatially weighted sum of the voltage gradient was determined at a point 2 cm from the sub-epicardium end of the heterogeneous multicellular fibre, along the fibre axis. The number of computed beats needed to reach convergence in ECG and action potential (AP) mathematical parameters, was determined by following at each beat, computed single cardiomyocyte AP and Ca^{2+} transient evolution at 1000 ms cycle length, starting from the model initial default conditions. Of note, the number of iterations needed to reach steady-state cannot be used to predict the number of action potentials necessary to reach biological steady-state. At first, AP duration decreased and Ca^{2+} transient amplitude increased to reach a constant value at the 250th beat (Supplementary material online, *Figure S2*; https://models.cellml.org/e/71). A value of 300 beats was chosen for all the tested conditions as it reflected stability of the modelling conditions. The healthy condition was modelled at 1000 ms cycle length, and tachycardia was modelled at 700 ms cycle length, that is commonly observed in COVID-19 patients[3] and at 500 ms cycle length.

To model cardiac response of COVID-19 patients with moderate hypokalaemia, external K^+ concentration has been decreased from 5.4 to 3.4 mM.

We reasoned that LQTS patients with major alterations in repolarization would not be prescribed QT lengthening compounds. Thus, we operated moderate modifications of the implicated currents to model LQTSs. For Type 1 LQTS, we reduced the conductance of the slow component of the delayed rectifier K^+ current (I_{Ks}) to 50% of the wild-type condition to mimic moderate loss-of-function of mutated *KCNQ1*-encoded channels, without any dominant negative effect usually associated with severe LQT.[49,50] Similarly, for Type 2 LQTS (LQT2), we reduced the conductance of the rapid component of the delayed rectifier K^+ current (I_{Kr}) to 50% of the wild-type condition to mimic moderate loss-of-function.[51] For Type 3 LQTS (LQT3), we reproduced the consequences of the ΔQKP1507-1509 mutant on *SCN5A*-encoded channel, $Na_v1.5$, with four-fold increase in the conductance of the late component of the Na^+ current.[52,53]

Effects of 3 μM HCQ have been chosen based on the serum concentration measured in COVID-19 patients treated with 600 mg/day.[10] Hydroxychloroquine effects on ion channels have been modelled as follows: 35% decrease of I_{Kr} conductance and 12% decrease of the conductance of the L-type Ca^{2+} current, $I_{Ca,\ L}$.[48] For AZM, data on serum concentrations from SARS-Cov2 patients are not available so far. Peak plasma AZM concentrations during oral dosing range from ≈0.4 to 1.1 μmol/L. However, plasma concentrations are misleading, as the drug accumulates within cells, achieving concentrations approaching 900 μmol/L in leukocytes and pulmonary tissue.[28] A previous study by the pharmaceutical sponsor, Pfizer Inc., reported similar accumulation of the drug in cardiac cells for mice receiving oral AZM (200 mg/kg day for 10 days), with ≈200-fold increase in concentration compared with plasma at Day 10.[54] Based on that, Yang *et al.*[28] used an *in vitro* concentration of 50 μM, which seems reasonable to estimate the effect on cardiac currents. Of Note, AZM has different effects with regard to acute (instantaneous) or 'chronic' 24 h exposure, regarding the Na^+ current. Acute exposure to AZM was shown to decrease both the peak sodium current and peak L-type calcium current. It also decreases the inward rectifier potassium current I_{K1}, and the delayed potassium currents I_{Kr} and I_{Ks}. In

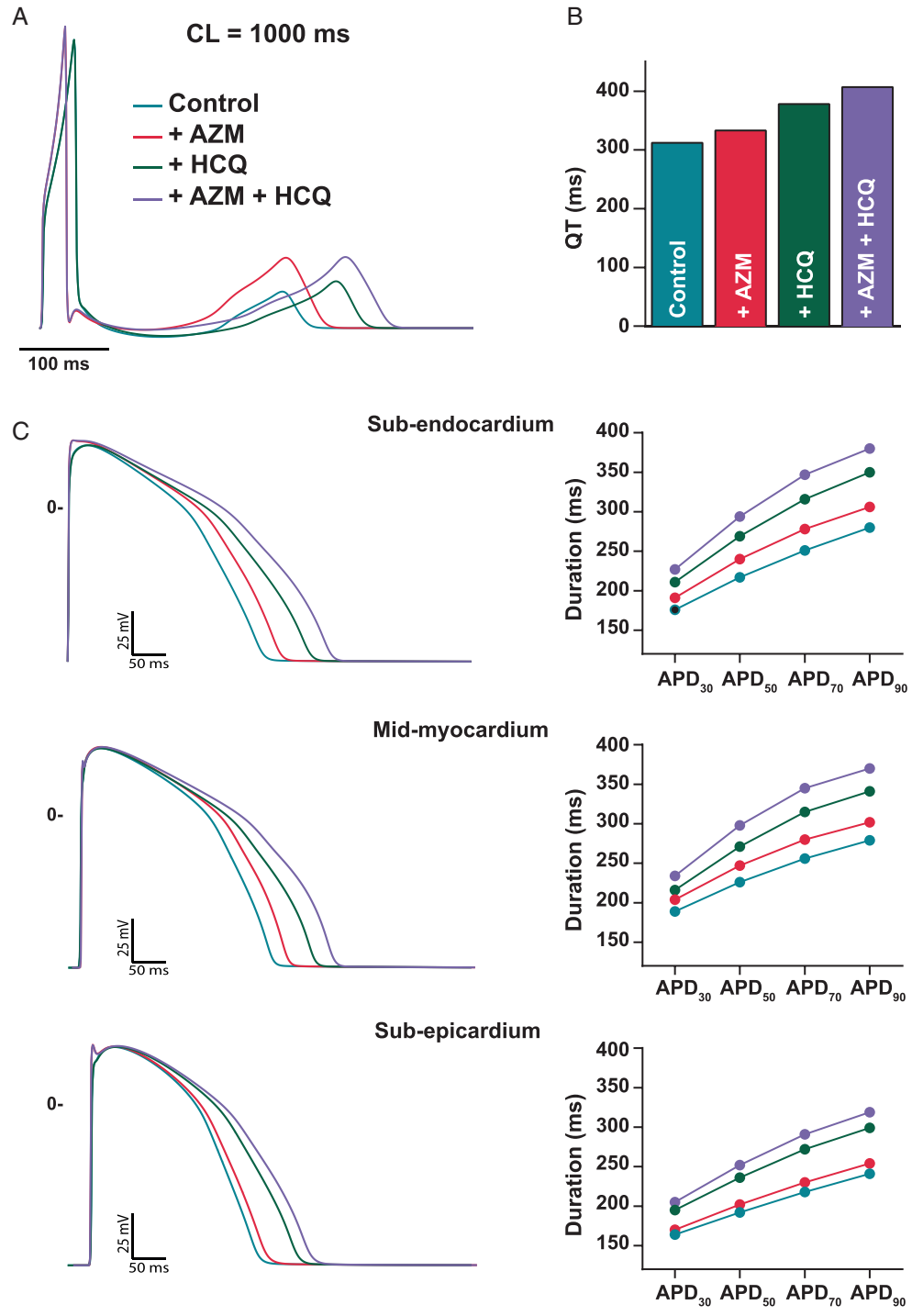

Figure 1 Hydroxychloroquine/azithromycin-induced prolongation of ventricular repolarization in a healthy heart (wedge *in silico* model). (*A*) Computed pseudo-ECG in control, AZM, HCQ, and AZM+HCQ conditions at 1000 ms of cycle length (CL). (*B*) QT interval measured in each condition. (*C*) Left: simulation of ventricular action potential from sub-endocardium (top), mid-myocardium (middle), and sub-epicardium (bottom) in control, AZM, HCQ, and AZM+HCQ condition. Right: quantification of action potential duration at 30% (APD_{30}), 50% (APD_{50}), 70% (APD_{70}), and 90% (APD_{90}) of repolarization in each condition. AZM, azithromycin; HCQ, hydroxychloroquine.

contrast, 24 h exposure increases the peak and late sodium currents. Unfortunately, the effects of 24 h exposure AZM on I_{K1}, I_{Kr}, I_{Ks}, and the L-type calcium current were not tested in this earlier report.[28] Interestingly, even in the case of acute exposure, Yang *et al.* showed an enlargement of QTc duration in mice (see *Figure* 2 of their publication), suggesting that the decrease in L-type calcium current is counterbalanced

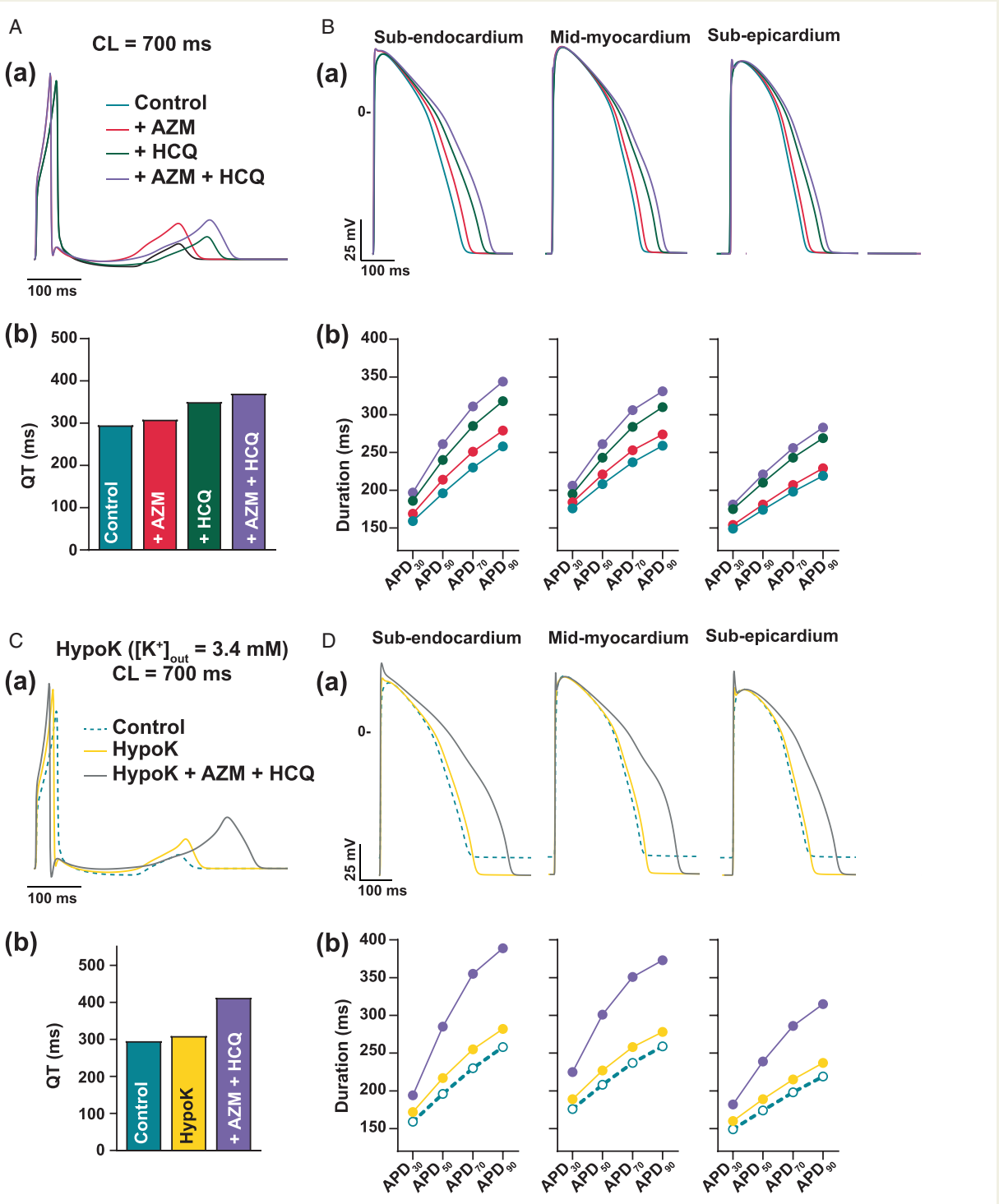

Figure 2 Hydroxychloroquine/azithromycin combination effects in COVID-19 patient model with tachycardia and hypokalaemia. (*A*) Tachycardia, (a) computed pseudo-ECG in control, AZM, HCQ, and AZM+HCQ conditions at 700 ms of cycle length. (b) QT interval measured in each condition. (*B*) (a) Simulation of ventricular action potential from sub-endocardium (left), mid-myocardium (middle), and sub-epicardium (right) in control, AZM, HCQ, and AZM+HCQ condition. (b) Quantification of action potential duration at 30% (APD_{30}), 50% (APD_{50}), 70% (APD_{70}), and 90% (APD_{90}) of repolarization in each condition. (*C*) Hypokalaemia, (a) computed pseudo-ECG in control, hypokalaemia (3.4 mM extracellular K^+), and hypokalaemia with AZM+HCQ conditions at 700 ms of cycle length. (b) QT interval measured in each condition. (*D*) (a) Simulation of ventricular action potential from sub-endocardium (left), mid-myocardium (middle), and sub-epicardium (right) in control, hypokalaemia and hypokalaemia with AZM+HCQ condition. (b) Quantification of action potential duration as in (B) (b), in each condition. AZM, azithromycin; HCQ, hydroxychloroquine.

Table 1 Drugs effects on pseudo-ECG parameters

	QRS (ms)							QT (ms)						
	Basal	+ AZM	+ HCQ	+AZM + HCQ	+AZM + HCQ + MEX	+ Dof.	+ Quinidine	Basal	+ AZM	+ HCQ	+AZM + HCQ	+AZM + HCQ + MEX	+ Dof.	+ Quinidine
Control	46	35	46	35	35	ND	ND	312	333	378	407	382	ND	ND
Tachycardia	47	35	47	35	35	50	48	297	310	352	372	353	410	470
HypoK	40	ND	ND	32	32	40	40	311	ND	ND	414	378	395	461
LQT1	46	ND	ND	35	35	50	48	307	ND	ND	388	368	433	506
LQT2	46	ND	ND	35	35	50	48	394	ND	ND	478	457	512	587
LQT3	46	ND	ND	35	35	50	48	337	ND	ND	487	433	466	538

Control at 1000 ms cycle length and other conditions at 700 ms cycle length.
AZM, azithromycin; Dof., dofetilide; ECG, electrocardiogram; HCQ, hydroxychloroquine; HypoK, hypokalaemia; MEX, mexiletine; ND, not determined.

or even exceeded by the decrease in I_{K1} (I_{Kr} and I_{Ks} being absent in adult mice). Because AZM is administered for several days in the COVID-19 context, we considered only reported 24 h effects of this compound on ion channels, as follows: 1.8-fold increase in sodium peak current conductance and 2.5-fold increase in late sodium current conductance. Of note, adding the other current alterations, based on their studied acute effects (65% reduction in L-type calcium current, 30% reduction in I_{Kr} and I_{Ks}, 66% reduction in I_{K1}) further increased QT duration (supplementary material online, *Figure S3*). Since the equivalence between acute and 24 h effects is hypothetical, we chose to keep the condition with the minimal effect (only clearly established 24 h effect of AZM on sodium channel). This point stresses out the importance of the characterization of longer application (at least 24 h) of a given molecule on the currents.

State-dependent effects of mexiletine (MEX) were modelled at therapeutic concentration (0.8–2 µg/mL)[55] by a 40% decrease in only the late sodium current conductance.[56]

Two antiarrhythmic drugs, that prolong QT interval and have been reported to induce TdP, were also tested as positive controls. Dofetilide is mostly active on I_{Kr} and I_{to} at ∼2 nM corresponding to the free plasma C_{max} concentration (factors applied: 0.45, 0.98, 0.98, 0.85, 0.98, and 0.95 to I_{Kr}, $I_{Ca,\,L}$, $I_{Na\ fast}$, I_{to}, I_{Ks}, and I_{K1} conductances, respectively).[56] Quinidine has a larger spectrum and was tested at its free plasma C_{max} concentration of ∼850 nM (factors applied: 0.3, 0.9, 0.98, 0.85, 0.9, and 0.95 to I_{Kr}, $I_{Ca,\,L}$, $I_{Na\ fast}$, I_{to}, I_{Ks}, and I_{K1} conductances, respectively).[56]

Combined effects (such as LQT mutation+AZM+HCQ+MEX) were obtained by applying each factor respective of each drug or condition to the appropriate conductance(s). Models were processed with C++ code.

Electrophysiological determinations

Pseudo-ECG time parameters were determined as previously described.[57] As expected, this model adapts to frequency by decreasing QT duration when frequency increases.[39]

As presented above, pseudo-ECG models are obtained from 1D strand of 165 cells reporting left ventricle transmural activity. For example, apex-to-base and right ventricle-to-left ventricle gradients are absent in this model. Therefore, generated pseudo-ECG time parameters are lower than human ECG values. QRS and QT durations are 46 and 312 ms, respectively, in this model compared with 90–100 and 370–440 ms in patients (i.e. –60 ms), suggesting that difference in QT between the model and patients is mainly due to the difference in QRS, not ST duration. For the sake of comparison, we arbitrarily added the empirical value of 60 ms to the model QT duration to obtain 'clinical-like' QT values (in *Figure 7*). QRS widening induced by HCQ may occur in COVID-19 patients. It is a slight median increase of 4 ms of borderline significance.[58] However, we kept this 60 ms value constant in every tested condition.

Arrhythmogenic risks were assessed by the repolarization time from APD_{30} to APD_{90} ($APD_{90\text{-}30}$) measured from the beginning of AP upstroke until 30% and 90% of repolarization as previously described[59] and by QT duration.[60] The breaks in the repolarization slope in early Phase 3 of the computed APs were considered as early afterdepolarizations (EADs) by analogy with the EAD originally defined as a depolarizing afterpotential that begins prior to the completion of repolarization and causes (or constitutes) an interruption or retardation of normal repolarization, in the *princeps* publication by Cranefield.[61] Data were analysed using R3.6.2 and GraphPad8.

Results

We started with the most general case of COVID-19 patients, presenting no arrhythmia risk factor that reduces the repolarization reserve. Thus, we first investigated the effects of HCQ or AZM alone and their combined effects on the ventricular repolarization on simulated 'normal' ECG. At a cycle length of 1000 ms, we observed that AZM alone induced a shortening of the QRS complex (−24%) and an increase in QT duration (+7%) due to the increased contribution of the peak and late sodium currents, respectively (*Figure 1A and B* and *Table 1*). Hydroxychloroquine alone induced a larger increase in QT duration (+21% vs. baseline) without affecting the QRS duration (*Figure 1A and B*). The combined AZM and HCQ synergistically prolonged the QT interval (30%; *Figure 1A and B*). These drugs target two different types of ion channels. Hydroxychloroquine reduces a repolarizing current (I_{Kr}), while AZM increases a depolarizing current (late I_{Na}). Their effects are thus more than additive on the action potential duration as described by previous studies.[62] Looking at specific cell levels, modelled action potentials from sub-endocardium (cell #19), mid-myocardium (cell #84), and sub-epicardium (cell #144) underwent major modifications when the effects of HCQ alone or combined with AZM were simulated (*Figure 1C*).

Because COVID-19 patients admitted in ICU are frequently tachycardic, we investigated the effects of the treatment at a faster rate

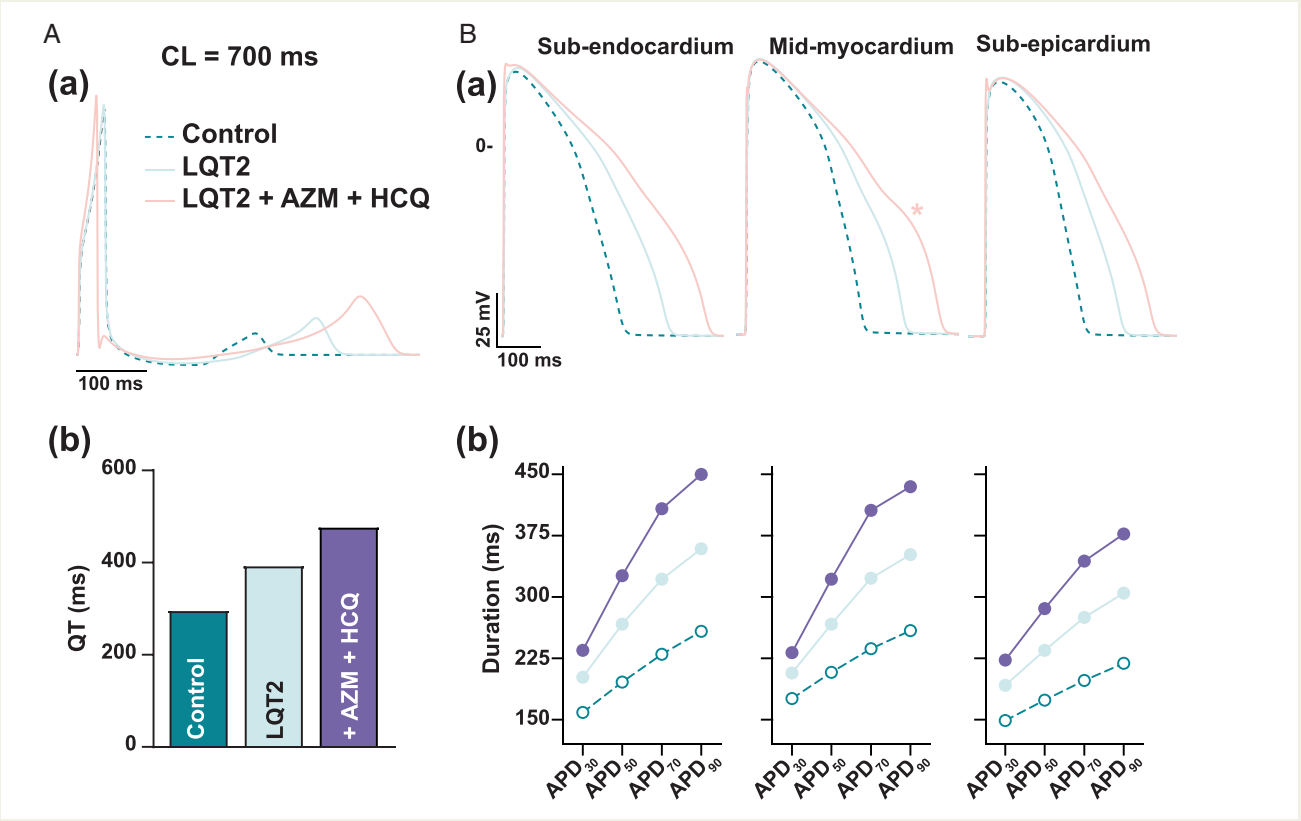

Figure 3 Arrhythmogenic effects of AZM+HCQ combination in long QT Type 2 model. (*A*) (a) Computed pseudo-ECG in control, long QT Type 2 (LQT2) modelled as a *KCNH2* haploinsufficiency, and LQT2 with AZM+HCQ condition at 700 ms of cycle length. (b) QT interval measured in each condition. (*B*) (a) Simulation of ventricular action potential from sub-endocardium (left), mid-myocardium (middle), and sub-epicardium (right) in control, LQT2, and LQT2 with AZM+HCQ condition. *: subthreshold early afterdepolarization. (b) Quantification of action potential duration at 30% (APD_{30}), 50% (APD_{50}), 70% (APD_{70}), and 90% (APD_{90}) of repolarization in each condition. AZM, azithromycin; HCQ, hydroxychloroquine.

(cycle length, CL = 700 ms). The resulting effects of the three treatments on pseudo-ECG and APs parameters were in the same range as at 1000 ms CL (*Figure 2A and B* and *Table 1*). When higher heart rate was tested (CL = 500 ms), similar results were observed (Supplementary material online, *Figure S4*).

Because hypokalaemia can precipitate acquired LQTS,[63] we investigated AZM and HCQ effects when a moderate hypokalaemia (3.4 mM of extracellular K^+) commonly observed in COVID-19 patients[64] was implemented in the model in addition to tachycardia. As shown in *Figure 2C*, hypokalaemia induced a QT prolongation (+5% compared with normokalaemia at 700 ms CL) and exacerbated the effects of the AZM+HCQ combination (+33% of increase in QT compared with +25% of increase in normokalaemia at 700 ms CL). Hypokalaemia also hyperpolarized the diastolic membrane potential of each cardiomyocyte layer (−99.2 mV in hypokalaemia vs. −86.9 mV in normokalaemia) leading to increased sodium channel availability. This increased availability caused QRS shortening. The combination of both drugs induced a triangulation of the AP shape as assessed by the prolongation of the repolarization time from APD_{30} to APD_{90} ($APD_{90\text{-}30}$; 195 vs. 110 ms with no treatment, in the sub-endocardium; *Figure 2D*), which is known to favour EADs.[65,66] In summary, the model suggests that COVID-19 patients with tachycardia and hypokalaemia, even 'sub-clinical', have to be closely monitored due to the potentiation of HCQ and AZM arrhythmogenic effects.

QT and AP duration lengthening were also observed when the reference drugs dofetilide and quinidine were applied (Supplementary material online, *Figures S5 and S6*). The deleterious effects of well-known arrhythmogenic drugs can be clearly identified. It appears that AZM+HCQ have similar effects as dofetilide, a high-risk torsadogenic drug. In summary, our results confirm the AZM+HCQ-induced QT prolongation observed in patients and validate the use of the model to investigate the arrhythmogenic consequences of drugs to treat COVID-19.

In order to validate the use of this model to predict arrhythmogenic susceptibility of patients with moderate LQTS, we first tested AZM and HCQ effects in an LQT2 model replicating hERG haploinsufficiency in normokalaemia. As expected, a 33% prolongation of the QT was obtained. AZM+HCQ combined effects further prolonged it by 21% vs. 'untreated' LQT2 condition (*Figure 3A*). In LQT2 conditions, AP repolarization relies mostly on I_{Ks}. As expected, the AZM-HCQ combined effects were major in the mid-myocardium where I_{Ks} is of small amplitude. Mid-myocardium $APD_{90\text{-}30}$, already prolonged by I_{Kr} decrease, was severely prolonged from 145 to 203 ms by AZM+HCQ treatment and associated with the

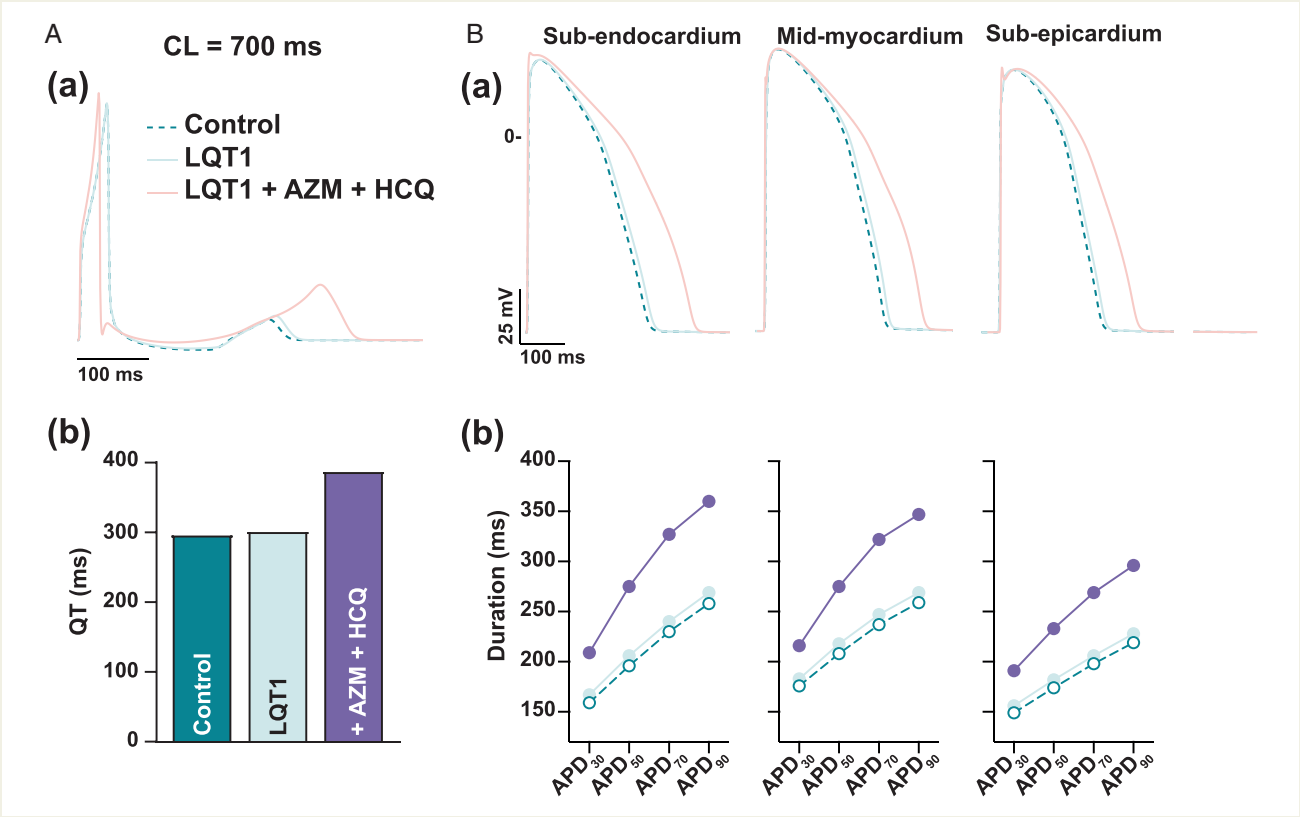

Figure 4 Hydroxychloroquine/azithromycin combination reveals arrhythmia susceptibility in asymptomatic long QT Type 1 model. (*A*) (a) Computed pseudo-ECG in control, long QT Type 1 (LQT1) modelled as a *KCNQ1* haploinsufficiency, and LQT1 with AZM+HCQ condition at 700 ms of cycle length. (b) QT interval measured in each condition. (*B*) (a) Simulation of ventricular action potential from sub-endocardium (left), mid-myocardium (middle), and sub-epicardium (right) in control, LQT1 and LQT1 with AZM+HCQ condition. (b) Quantification of action potential duration at 30% (APD_{30}), 50% (APD_{50}), 70% (APD_{70}), and 90% (APD_{90}) of repolarization in each condition. AZM, azithromycin; HCQ, hydroxychloroquine.

occurrence of a subthreshold EAD (*Figure 3B*). Again, AZM+HCQ treatment had effects in the same range as those observed with dofetilide (Supplementary material online, *Figures S5 and S6*). In the same conditions, quinidine application led to more pronounced QT prolongation and EADs particularly at the mid-myocardium level. These sets of data show that the ORd model replicated the impact of proarrhythmic drugs on LQT2 AP and ECG. These results confirm the absolute proscription of the use of such proarrhythmic drugs in COVID-19 patients with baseline long QT.[67–69]

Then, we used the model to predict the effects of AZM and HCQ in the context of a sub-clinical QT prolongation as seen in parents of patients with autosomal recessive Jervell and Lange-Nielsen LQTS, for instance.[70] Despite a 50% reduction in I_{Ks} amplitude, a minimal 3% prolongation of the QT duration was observed (*Figure 4A*). However, the combination of AZM and HCQ induced a 26% increase in QT duration (vs. 'untreated' LQT1 condition) as well as AP prolongation (*Figure 4*). This approach suggests that COVID-19 patients with primary moderate hypokalaemia or asymptomatic LQT1 have a slightly higher risk to develop drug-induced arrhythmias when treated with AZM and HCQ than patients without these co-morbidities (+11% and +4% QT prolongation in hypokalaemia and LQT1, respectively, compared with QT values of 'treated' 'normal' ECG at the same heart rhythm). These patients have to be followed closely and additional preventive anti-arrhythmic therapy might be proposed in this case.

As AZM increases the late component of the sodium current, we also investigated the effects of the combined therapy in a model in which the late component of the Na^+ current was already increased i.e. in the model replicating LQT3. A 13% QT prolongation was obtained, to a lesser extent than in the LQT2 condition, though. However, a dramatic QT prolongation of 44% was induced by AZM+HCQ treatment (*Figure 5A*). At the 'cellular level', combining both drugs effects favoured AP triangulation ($APD_{90\text{-}30}$ duration increased from 102 to 195 ms) and occurrence of EADs in mid-myocardium, close to what was obtained with the LQT2 model (*Figure 5B*).

Since it appears that the ORd model confirmed the observed and expected results regarding HCQ and AZM effects on ECG, we used the model to predict the effect of mexiletine treatment. Mexiletine, a well-known anti-arrhythmic drug used in LQTS patients was proposed to be associated with HCQ and AZM treatment of COVID-19 patients to limit excessive QT prolongation.[71,72] As shown in *Figure 6*, mexiletine reversed AZM+HCQ-induced QT prolongation in all tested conditions (+19% vs. +25% in tachycardia, +22% vs. +33% in

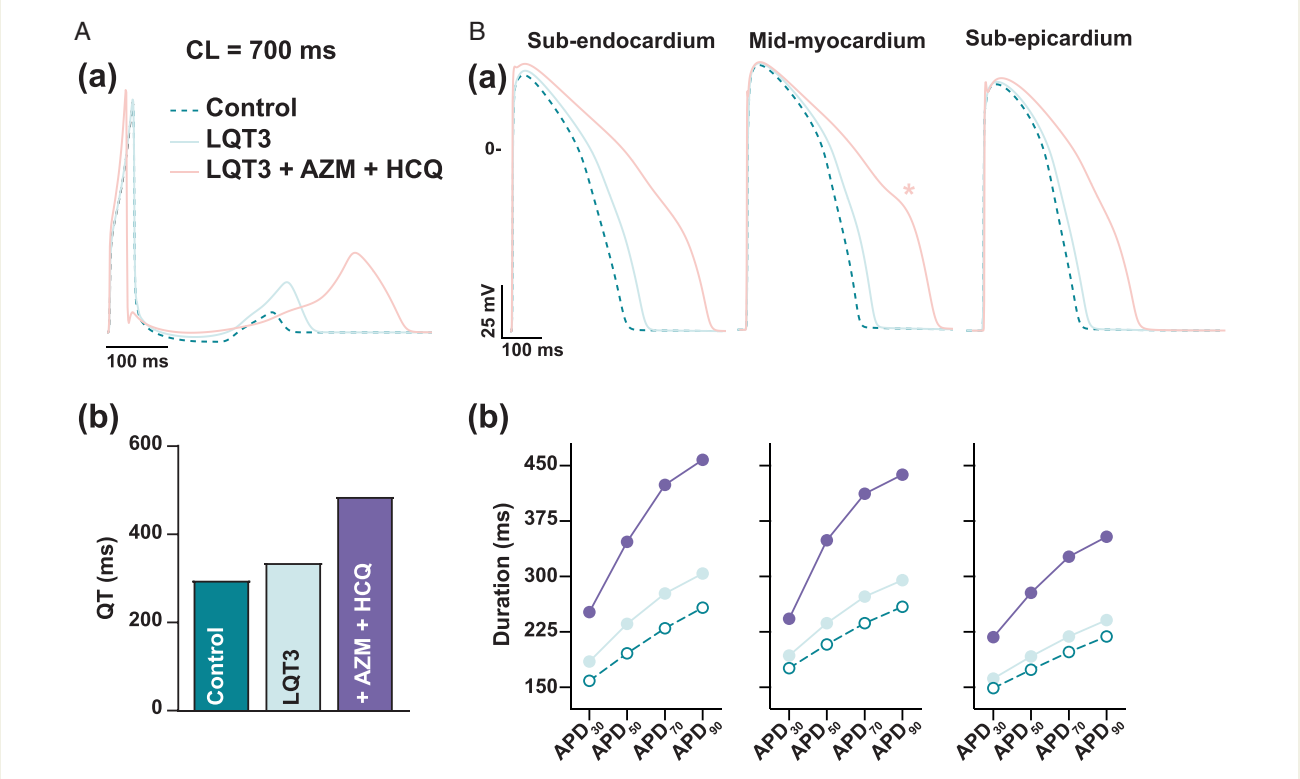

Figure 5 Arrhythmogenic effects of AZM+HCQ combination in long QT Type 3 model. (*A*) (a) Computed pseudo-ECG in control, long QT Type 3 (LQT3) modelled as a four-fold increase in persistent sodium current, and LQT3 with AZM+HCQ condition at 700 ms of cycle length. (b) QT interval measured in each condition. (*B*) (a) Simulation of ventricular action potential from sub-endocardium (left), mid-myocardium (middle), and sub-epicardium (right) in control, LQT3 and LQT3 with AZM+HCQ condition. *: subthreshold early afterdepolarization. (b) Quantification of action potential duration at 30% (APD_{30}), 50% (APD_{50}), 70% (APD_{70}), and 90% (APD_{90}) of repolarization in each condition. AZM, azithromycin; HCQ, hydroxychloroquine.

hypokalaemia, +16% vs. +21% in LQT2, +20% vs. +26% in LQT1, and +28% vs. +45% in LQT3 model). In hypokalaemia, LQT2 and LQT3 models, mexiletine reduced the AZM+HCQ-induced EAD susceptibility in mid-myocardium ($APD_{90\text{-}30}$ of 131 vs.148 ms, 186 vs. 203 ms, and 159 vs. 195 ms for in hypokalaemia, LQT2, and LQT3 models, respectively). Of note, the model predicts that mexiletine supplementation to shorten the prolonged QT has a mild but not negligible effect. Moreover, the model may be robust enough to evaluate the combined effects of new additional drugs (with known effects on ion channels) to limit AZM+HCQ arrhythmogenic consequences.

Figure 7 summarizes the QT duration values obtained at 700 ms cycle length. The ORd transmural wedge model values are arbitrarily transposed to clinical-like values by adding 60 ms (right *Y*-axis). A QTc cut-off of 500 ms is clinically considered as pathological.[67–69] At 700 ms of cycle length, the corresponding absolute QT duration according to Bazett's formula is 418 ms.

Discussion

Our study confirms that treating COVID-19 patients with HCQ and AZM drugs has, in most patients, little impact on QT duration[73] and does not induce any substrate prone to arrhythmia. However, in clinical conditions in which the repolarization reserve is reduced, the model predicts larger ECG impairments including QT >418 ms at 700 ms of cycle length, corresponding to QTc > 500 ms (*Figure 7*). Such dramatic QT prolongations are potentially enabling the occurrence of life-threatening events, such as ventricular fibrillation. In addition, the model allows the dissection of the relative contribution of each drug to the establishment of pro-arrhythmic conditions, as well as their synergic effects to the mechanisms involved. We also show that, mexiletine can limit only partly the dramatic increase in QT duration for patients with tachycardia, hypokalaemia, or reduced conduction reserve, but can bring it back to manageable duration for the mildest phenotypes. These results are in agreement with observations reported by Badri *et al.*[74] after mexiletine treatment on acquired-LQT syndrome patients. The use of lidocaine, another class I antiarrhythmic drug has shown some benefits in a COVID-19 patient treated with AZM and HCQ.[75] Therefore, the ORd model may be used to evaluate the potential impact of additional drug, with known effects on ion channels, to limit the arrhythmogenic risks.

There is currently an explosion of proposed therapies for treating the virus but none of them have clearly demonstrated their efficacy.[76] Among these therapies, HCQ combined with AZM is still being used

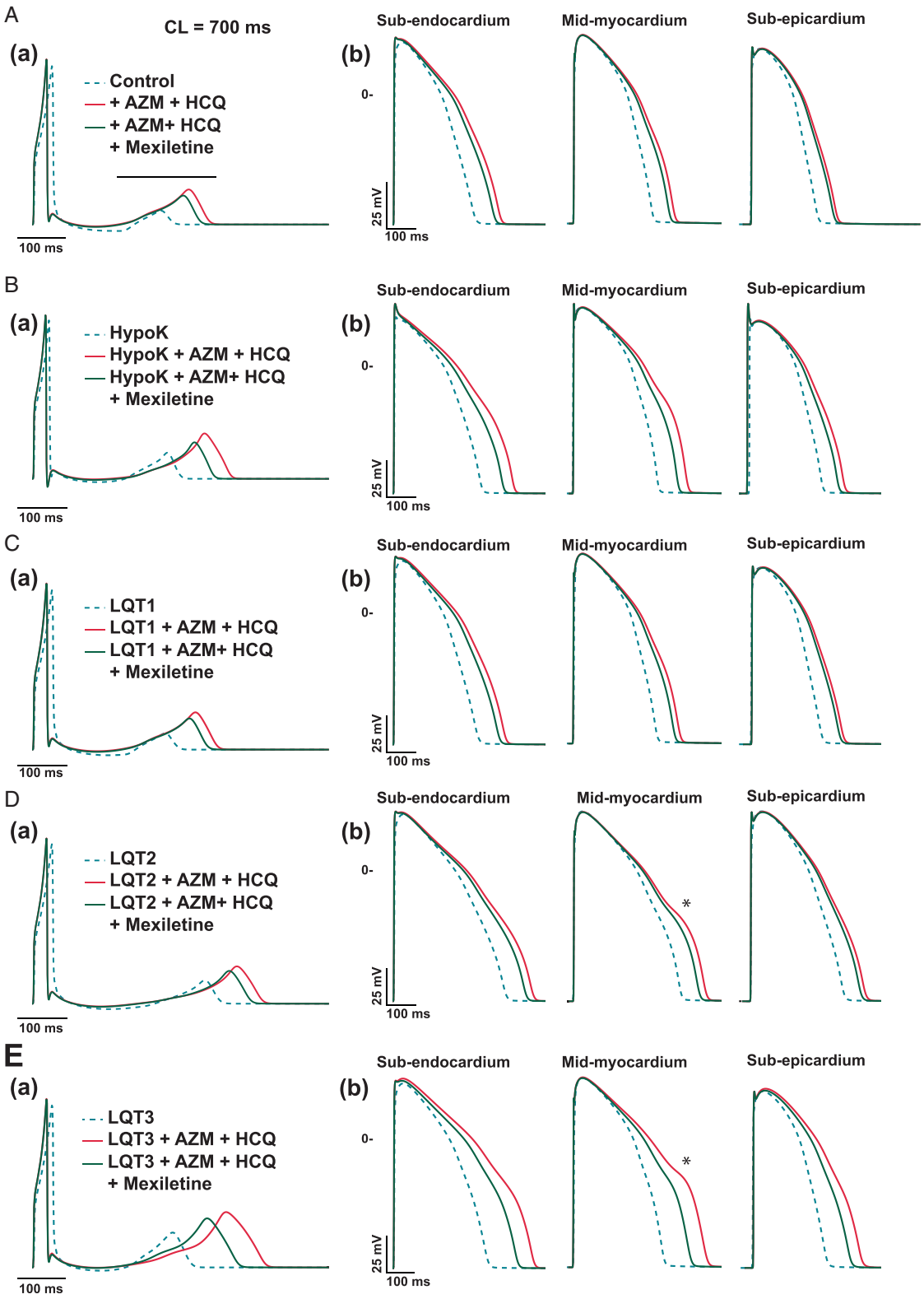

Figure 6 Mexiletine partially limits AZM+HCQ-induced QT prolongation. Combination of mexiletine with AZM+HCQ limits increase in QT interval in pseudo ECG (a) and simulated ventricular action potential (b) prolongation in tachycardia (*A*), hypokalaemia (*B*), LQT1 (*C*), LQT2 (*D*), and LQT3 (*E*) conditions compared with the same condition without mexiletine. AZM, azithromycin; HCQ, hydroxychloroquine.

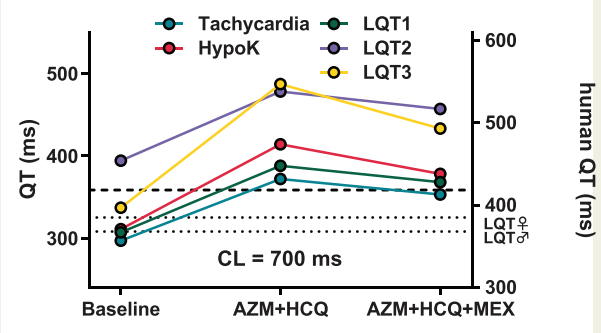

Figure 7 ORd model QT transposed to clinical human QT. In all conditions, converted AZM+HCQ QT values exceed (right *Y*-axis) the 418 ms cut-off (dashed line) and the use of mexiletine allows a partial reversion close to the cut-off value in tachycardia alone or combined with hypokalaemia or LQT1 condition. AZM, azithromycin; HCQ, hydroxychloroquine; ORd, O'Hara-Rudy.

based on *in vitro* studies indicating their ability to inhibit virus-cell fusion[7–9] and despite accumulation of studies questioning their clinical efficacy, the topic had been thoroughly debated.[12–16] A major concern of this therapy has been the risk of QT prolongation and TdP. The proarrhythmic mechanism of HCQ is thought to be due to its ability to inhibit hERG potassium channel and L-type calcium channel, which can result in EAD triggered activity.[48] Association of well-timed EAD and QT prolongation results in TdP. The proarrhythmic mechanism of AZM is thought to be due to its ability to increase cardiac sodium current and promote intra-cellular sodium loading.[28] Obviously, clinical decision cannot rely on the results obtained with this ECG model, but, by comparing the effects obtained with AZM+HCQ, and two proarrhythmic drugs, it can be suspected that the treatment has deleterious effects *in vivo*. Indeed, based on the proposed mechanisms we confirmed, using this *in silico* model, recent reports indicating QT prolongation[73,77] and high risk of TdP[78] in COVID-19 patients treated with HCQ and/or AZM. The discrepancy between occasional reports of QT prolongation and life-threatening arrhythmias triggered by HCQ and AZM and the absence of QT prolongation effects in large population studies (especially with AZM[34]), is probably due to the necessity, for triggering arrhythmia, of the combination of factors such as tachycardia, hypokalaemia, and subclinical LQTS as substrate.

More than 280 drugs have been reported to induce QTc prolongation.[79] Among them several are antiarrhythmic drugs, but also non-cardiovascular drugs, that are widely used in ICU.[80] Clear recommendations have been established to avoid their administration to patients with symptomatic and well-established congenital LQTS. In addition, I_{Kr}, I_{Ks}, $I_{Ca,\ L}$, $I_{Na\ late}$, and more generally Ca^{2+} homeostasis, are differentially impaired in various cardiopathies and cardiomyopathies frequently associated with aging, and also in hypoxia, much more frequent conditions in hospitalized COVID-19 patients. This is of concern, especially since $I_{Na\ late}$ increase, most frequently associated with these acquired diseases, appears to lead to severe ECG changes (LQT3).

Regardless of genetic aspects or pre-existing chronic pathologies, clinical case series have also identified risk factors for drug-induced LQTS including hypokalaemia as commonly observed in COVID-19 patients.[81] Hypokalaemia prolongs QT and is a risk factor for drug-induced LQTS. In addition to direct consequences on I_{Kr} current,[82,83] hypokalaemia may activate CaMKII leading to an increase in late sodium current and further prolongation of ventricular repolarization.[84] Moderate to severe hypokalaemia has been reported in COVID-19 patients.[64] SARS-CoV-2 virus invades cells through binding to angiotensin I converting enzyme 2 (ACE2) that enhances ACE2 degradation. The final effect of this degradation is a continuous renal K^+ loss that makes it difficult to correct hypokalaemia.[64] Noteworthy, low levels of potassium have been correlated with QTc > 500 ms occurrence in COVID-19 patients under HCQ and AZM medication.[37] Consistent with these observations, this model emphasizes the fact that kalaemia of COVID-19 patients has to be followed very carefully, particularly in case of medication with drugs such as HCQ or AZM. More generally, this model could be used to evaluate in a pre-clinical approach, the risk of drug-induced QT prolongation in this context. In addition to electrolyte imbalance, there is also a greater prevalence of risk factors among COVID-19 patients in ICU, including older age, presence of underlying heart disease, and co-treatment with other QT prolonging medications.

With the possibility that a significant proportion of the world population may receive SARS-CoV-2 drugs with torsadogenic potential, the risk to treat patients with asymptomatic and undiagnosed LQTS is increasing. These patients have a QTc duration in the limit of the general population variability and are not identified as such. Indeed, in a recent study, patients with extreme QTc prolongation when treated with HCQ and AZM, presented a baseline QTc ~431 ms only, within the normal QTc range.[37] As modelled in the present study, cardiomyocytes harbouring mutations leading to haploinsufficiency in *KCNQ1* may present very minimal action potential prolongation because of a normal I_{Kr},[85] but I_{Kr} blockers such as HCQ, can lead to marked action potential prolongation in limited repolarization reserve. All guidelines for QT management in COVID-19 context[67–69] recommend to avoid QT prolonging drugs in individuals with a QTc > 500 ms due to a two-fold to three-fold increase in risk for TdP.[86] Nevertheless, those asymptomatic patients might receive these drugs based on this criterion. As modelled in this study, despite the absence of QT prolongation in baseline conditions because of a normal I_{Kr}[85] and regardless of the origin of low repolarization reserve, these patients are at high risk of TdP when I_{Kr} blockers such as HCQ are used. However, the model shows that, in a borderline condition such as moderate LQT1, mexiletine can limit to some extent the deleterious effects of AZM and HCQ. Therefore, we propose that the ORd model can be used to evaluate the potential impact of other additional drugs, with known effects on ion channels, which may be used in the future to limit arrhythmogenic risk of COVID-19 therapies.

In summary, the ORd model appears to be an easy-to-use tool to assess off-label drug arrhythmia potential in different conditions representative of COVID-19 patients at risk for arrhythmia and life-threatening TdP.

Limitations

We used the original ORd model based on its more realistic conductance values compared with others. This model may underestimate I_{Ks} amplitude even if obtained from human cardiomyocytes.[87] In

some rare cases (heterozygous non-dominant-negative LQT1 mutations), a minimal reduction (<50%) of the channel activity leads to severe QTc prolongation (very minor cases, *cf.* for instance[88]). The model we use is simple, robust, and incorporates pseudo ECGs but not inter-individual variability, to remain affordable in time and resources. Since the model does not include the population variability (e.g. due to genetic background), it cannot reproduce the LQTS phenotype heterogeneity. Other studies focusing on single-cell AP, adjusted I_{Ks} amplitude to compensate for this insufficiency leading to more severe LQT1 phenotype.[89] It would be interesting to try optimizing pseudo-ECG models using the same strategy.

One-dimension strip of 165 cardiomyocytes simulates only the transmural gradient. Apex-to-base and right-to-left gradients are absent in this model. There are 3D models but (i) they are highly computationally demanding thus requiring simpler alternative approaches to model single-cell action potential[90] and (ii) they are less realistically adaptive because each current is not individually modelled. Therefore, we preferred to use a 1D-model in which precise biophysical equations representing the biological currents can be finely tuned to model the drug effects at the AP then ECG levels. Thus, the resulting caveat is the lower QT duration values. In order to allow translational approach, we suggest adding an empirically estimated value of 60 ms. The calculated QT values can then be roughly compared with clinical ECG values. In addition, T wave shape results more from regional heterogeneity than from transmural gradients.[91] As another limit of the 1D-model, it cannot simulate changes in T wave amplitude.

In this study, we investigated potential effects of drugs prescribed to patients with COVID-19 on AP with reduced repolarization reserve, in order to detect any arrhythmogenic substrate. To do so, we used well-defined conditions with 'pure' repolarization reserve decrease such as LQT syndrome with various genetic origins. Cardiopathies and cardiomyopathies frequently associated with aging, and in hypoxia, are much more frequent conditions in hospitalized COVID-19 patients. However, instead of adding another condition, generic for these pathologies, which is difficult to establish since conductance decreases are not the same for all the pathologies,[92] conditions with 'pure' repolarization-reserve decrease as LQT syndromes were preferred. Similarly, the complex modifications induced by systemic inflammation and oxidative stress observed in COVID-19 patients have not been introduced at the level of the ion currents in the modelling. Such complex alterations of expression and/or activity of ion channels are hardly quantifiable and cannot be mimicked. In any case, it can be suspected that the addition of pre-existing pathologic conditions and COVID-19-related modifications would exacerbate the arrhythmia susceptibility.

The effects of adrenergic stimulation were not evaluated for the following reason. An observational study of 138 patients affected by COVID-19 reported moderate tachycardia with a median heart rate of 88 b.p.m.[3] indicating that the adrenergic tone is not high in those patients. Thus, in this study, we evaluated, during moderate tachycardia, the theoretical effect of the drugs on AP with reduced repolarization reserve, in order to detect any arrhythmogenic substrate. Interestingly, a very recent work, complementary to ours, used a modified version of the ORd model to study the β-adrenergic receptor stimulation on the cellular proarrhythmic effects of CQ and AZM, at the single AP level.[93] In this article, Sutanto and Heijman suggest that sympathetic stimulation limits drug-induced APD prolongation. Therefore, at least for CQ and AZM, the unstimulated situation that we studied may represent the most critical situation.

It has to be mentioned that the ECG ORd model is conservative. Arrhythmogenic mechanisms such as triggered activities are hardly induced. Indeed, significant impairment of the Ca^{2+} current window was needed to induce repolarization failure in the recent study of Sutanto and Heijman.[93] However, the deleterious effects of arrhythmogenic drugs can be clearly identified with the ECG model, namely EADs and QT lengthening.

Gender differences, resulting from multiple intersecting processes implying complex regulations of ion channels, cannot be easily modelled and was not investigated in this study. This would be indeed another improvement of the model.

Supplementary material

Supplementary material is available at *Europace* online.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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